This is to certify that the

thesis entitled

KIDNEY FUNCTION OF THE PROGENY OF RATS WITH STREPTOZOTOCIN-INDUCED DIABETES

presented by

Ellen Christine Rolig

has been accepted towards fulfillment
of the requirements for

M.S. degree in Nutrition

Jerry J. Bond
Major professor

Date 8/10/79

O-7639

KIDNEY FUNCTION OF THE PROGENY OF RATS WITH STREPTOZOTOCIN-INDUCED DIABETES

By

Ellen Christine Rolig

A THESIS

Submitted to

Michigan State University

in partial fulfillment of the requirements

for the degree of

MASTER OF SCIENCE

Department of Food Science and Human Nutrition

1979

ABSTRACT

KIDNEY FUNCTION OF THE PROGENY OF RATS WITH STREPTOZOTOCIN-INDUCED DIABETES

By

Ellen Christine Rolig

Effects of gestational diabetes on development of kidney function in the progeny were determined *in vitro* and *in vivo*. Body and kidney weights of pups of diabetic mothers (PDMs) were no different than those of control mothers (PCMs) at 1, 5, or 10, but were lower at 28 days of age. Generally, the ability of renal cortical slices from 1, 5, 10, and 28 day old PDMs to accumulate p-aminohippurate (PAH), n-methylnicotinamide (NMN), α-methylglucoside (αMG), or aminoisobutyric acid (AIB) was similar to that of PCMs. Renal ammoniagenesis was enhanced in 1 and 5 day old PDMs but declined to control values at 10 and 28 days of age. Renal gluconeogenesis was enhanced only in 5 day old PDMs. Urinary response to a water or isotonic saline load by PDMs was no different than that by PCMs at 5 or 10 days of age. In contrast, 10 day old but not 5 day old PDMs excreted more urine, total solute, and sodium in response to hypertonic saline than age matched PCMs. These observations indicate that gestational diabetes selectively modified renal functional development.

Dedicated to my parents

HELEN AND GEORGE SHARP

and to my husband

STEVEN ROLIG

for unconditional love
and encouragement

ACKNOWLEDGMENTS

I am deeply grateful to Dr. Jenny Bond for her advice, encouragement, and constructive criticism during all phases of this study. My sincere thanks are extended to Dr. Jerry Hook for his invaluable guidance and his insistance on hard work and excellence. I would also like to thank Dr. David Rovner and Dr. Dale Romsos for their suggestions and critical evaluation of this work. A special thanks are due to my fellow graduate students, Bill Evers, Sue Ford, and Robin Goldstein, for encouragement, advice, and personal support throughout the course of this study.

I would like to acknowledge Dr. John Gill and Dr. Clyde Anderson for advice concerning statistical analysis of the data and Marty Bond for his assistance with graphic illustrations.

The financial support provided by Dr. Jenny Bond and the Department of Food Science and Human Nutrition is gratefully acknowledged.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Previous experiments have shown a direct relationship between nutritional status and kidney development in the fetus and neonate. Postnatal development of kidney structure and function in rats can be enhanced by pre- and postnatal overnutrition (Winick and Noble, 1966; Solomon and Capek, 1972; Bond et al., 1977) and retarded by pre- and postnatal malnutrition (Hall and Zeman, 1968; Allen and Zeman, 1971; 1973a; 1973b; Bond et al., 1977; Goldstein et al., 1979).

The nutritional status of the fetus and the infant of the diabetic mother appears to be different from that of a normal infant. Evidence to date suggests that the fetus of the diabetic mother is hyperinsulinemic (Steinke and Driscoll, 1965). Fetal hyperinsulinemia could be induced by excess glucose and amino acids available to the fetus of the pregnant diabetic (Osler and Pederson, 1960). The resulting infant of the diabetic mother (IDM) has a number of nutritional problems. Ten to thirty percent of IDMs are large for gestational age and 40-50% develop neonatal hypoglycemia (Persson, 1975; Kitzmiller et al., 1978). Neonatal hypoglycemia appears to be caused by a combination

of high plasma insulin concentration (Block et al., 1974) and an inappropriately small pancreatic glucagon response to the fall in blood glucose (Johnston and Bloom, 1975). The extent of this nutrient-hormonal imbalance in the fetus depends on the severity of the maternal diabetes (Farquhar, 1976). Consequently, IDMs are a heterogeneous group of infants with features of both over and under nutrition (Farquhar, 1976).

There is some indirect evidence that renal function is impaired in IDMs. IDMs are more likely than normal infants to develop metabolic acidosis (Lowery et al., 1954; Segal et al., 1957; Kitzmiller et al., 1978), to excrete greater quantities of electrolytes, water and nitrogen (Osler, 1960; Cook et al., 1960), and to develop hypocalcemia and hyperphosphatemia (Zetterstrom and Arnhold, 1958; Kitzmiller et al., 1978). While the renal glomerulus appears to develop normally in fetuses of diabetic women (Cassady et al., 1975; Naeye, 1975), recent data suggest that the renal tubules do not (Sokol and Hall, 1977). To date the direct effects of maternal diabetes on pre- and postnatal development of kidney function have not been investigated. Therefore, this study was undertaken to evaluate the effects of gestational diabetes in rats on the renal functional development of the progeny.

Renal Development

Anatomically, the kidneys of most mammals mature gradually throughout gestation and during early neonatal life. Nephron development begins in the juxtamedullary region and progresses toward the capsule (Baxter and Yoffey, 1948; Potter, 1965; Speller and Moffat, 1977). In humans, the number of new nephrons increases between six and thirty-six weeks of gestation. Maturation of the kidney in the newborn consists of growth and development of nephrons present at birth (Potter, 1965). Human fetal kidneys have nephrons at various stages of development up until 36 weeks of gestation when all nephrons have morphological characteristics of the adult type (Potter, 1965). Although full term human newborns have the same number of glomeruli and tubules as the adult, these are not structurally mature. Fetterman and co-workers (1965) examined the development of glomeruli and proximal tubules from birth to adulthood in human kidneys and found that all parts of the nephron increase in size and complexity with increasing age. The most striking of these changes involves the development of the convolutions of the proximal tubules and the formation and elongation of the loop of Henle. In contrast, kidneys of newborn rats have one third the number of nephrons present in adult kidneys and these are primarily juxtamedullary nephrons (McCrory, 1972; Speller and Moffat, 1977). Postnatal nephrogenesis in the rat occurs

primarily in the cortex by differentiation of successive layers of nephrons from the peripheral nephrogenic zone (Speller and Moffat, 1977). By 2 weeks after birth the number of nephrons has doubled and by four weeks, it has tripled (Kittelson, 1917). As the number of nephrons increases, the tubules lengthen and the interlobular arteries elongate to supply glomeruli (Speller and Moffat, 1977).

The development of kidney function is closely associated with, though not entirely dependent on, anatomical maturation. While kidney function in the healthy newborn of most species is adequate for survival and growth, it may be inadequate under stress (McCance and Wilkinson, 1947; McCance et al., 1954; Falk, 1955). Newborns when compared with adults have less ability to excrete a water, solute, acid or alkali load (Falk, 1955; Goldstein, 1970), less ability to concentrate urine in the presence of dehydration (Heller, 1947; Falk, 1955), a decreased response to antidiuretic hormone (ADH) (Heller, 1944; 1952; Falk, 1955), and both a decreased extraction of p-aminohippurate (PAH) (Horster and Lewy, 1970) and a decreased maximal tubular capacity for PAH (Hook et al., 1970). In addition, glomerular filtration rate (GFR) and renal blood flow (RBF) are directly related to age; both are lowest at birth and progressively increase to adult values (Potter et al., 1969; Horster and Valtin, 1971; Leake et al., 1977).

Renal blood supply and distribution influence kidney function and development. The neonatal kidney receives a lower percent of the cardiac output than the adult kidney (Kleinman and Reuter, 1973). Additionally, the distribution of blood within the neonatal kidney is different than that observed in the adult (Jose et al., 1971). This is due, in part, to a high renal vascular resistance present at birth (Assali et al., 1968; Gruskin et al., 1970). After birth there is a decrease in renal vascular resistance followed by an increase in renal blood flow associated with changes in the distribution of flow within the kidney. The greatest flow is supplied to the mature nephrons. Thus, in the immature kidney, the nephrons located in the inner cortex would receive the highest flow while the flow to the outer cortex would be low at birth and would increase with age (Jose et al., 1971; Kleinman and Reuter, 1973; Olbing et al., 1973).

Renal Tubular Transport Function

Renal tubular transport functions such as reabsorption and secretion depend on both the morphological and biochemical development of the specific cells involved, in particular, proximal tubular cells. In the adult kidney, these processes have been elucidated largely with the use of micropuncture techniques (Pitts, 1976). Since the immature kidney contains nephrons at various stages of

development (Fetterman et al., 1965), micropuncture techniques are inappropriate for the study of the development of renal tubular transport functions. For this reason, development of tubular function must be analyzed with more integrative techniques. One such technique is the kidney slice technique.

The slice technique as first described by Cross and Taggart (1950) has been used for many in vitro studies of renal transport processes and metabolism. Thin renal cortical slices incubated in an appropriate medium in an oxygen atmosphere will accumulate a number of compounds. Accumulation of compounds by renal cortical slices is expressed as the tissue concentration of material divided by the medium concentration or as the slice/medium (S/M) ratio or tissue/medium (T/M) ratio. If the S/M ratio exceeds one, it is thought to indicate an active transport process. Alternatively, renal cortical slices incubated with precursors will synthesize products such as glucose and ammonia. The kidney slice technique has both advantages and disadvantages. Advantages include the elimination of unwanted changes in GFR, RBF, and extra-renal factors, the precise control over incubation conditions, and the presence of many intact kidney cells. Disadvantages include the presence of both proximal and distal tubules in renal cortical slices and the absence of a continuous filtration process in the slice preparation. Additionally,

S/M ratios indicate the net accumulation resulting from the influx, the efflux, and nonspecific binding. Thus S/M ratios may indicate nonspecific binding and/or peritubular transport in conjunction with or rather than luminal transport (Berndt, 1976).

Experimental data from both animals and humans indicate renal tubular function in the newborn is different than in the adult. The immature kidney when compared to the adult kidney has a lower percent of the glomerular filtrate reabsorbed by the tubules (McCrory, 1972), a lower intrinsic sodium transport capacity (McCrory, 1972), a lower threshold for bicarbonate reabsorption (Edelmann et al., 1967), a lower capacity to reabsorb sugar (Segal et al., 1973a), and a lower capacity to secrete organic acids and bases (Rennick et al., 1961; Kim et al., 1972; Hook, 1974).

Reabsorption of Glucose

The renal mechanism for glucose reabsorption has been characterized as an active transport system of limited capacity located in the proximal tubule. It is sodium dependent, phlorizin inhibited, and highly specific for sugar substrates such as glucose, galactose, xylose, fructose, and non-metabolizable sugars such as αmethyl-D-glucopyranoside (αMG) (Pitts, 1976). The studies on renal glucose reabsorption during development are limited.

Tudvad (1949) compared sugar reabsorption among premature babies, full term babies, and adults and found that the reabsorption is low at birth and increases proportionately with age. *In vitro* experiments suggest that the active transport process for glucose in the proximal tubular cell in the immature kidney is different from that in the mature kidney (Kolinska, 1970; Segal *et al.*, 1971; 1973a; 1973b). Since glucose uptake is an active transport process, proximal tubular cells can accumulate glucose against a concentration gradient and can produce intracellular/extracellular glucose concentration ratios greater than one. Accordingly, the uptake and efflux of αMG by renal cortical slices have been characterized in rats (Segal *et al.*, 1971; 1973a), rabbits (Kolinska, 1970), and humans (Segal *et al.*, 1973b). Until recently, the interpretation of these data has been hampered by limitations inherent to the *in vitro* slice technique. Specifically, it was not known whether the uptake of αMG occurred at the peritubular or the luminal membrane. Current evidence indicates that αMG does not interact with the peritubular side but that it is transported into the proximal tubular cell via a glucose receptor at the brush border (Naftalin, 1970). Furthermore, the kinetics of αMG transport in the renal cortex slices reflect the kinetics of the glucose carrier at the brush border (Turner and Silverman, 1977).

Immature kidneys appear to have a lower capacity to reabsorb sugars than adult kidneys (Segal et al., 1973a). Cortical slices from kidneys of one day old rats were unable to actively transport αMG as indicated by αMG S/M ratios less than one. Slices from five day old rats concentrated αMG slightly and as the rats increased in age, the ability of kidney slices to accumulate αMG also increased. Renal cortical slices from fifteen day old rats accumulated the same amount of αMG as those from adults (Segal et al., 1973a).

Reabsorption of Amino Acids

The renal reabsorptive mechanisms for amino acids similarly involves active transport into proximal tubular cells. There are at least three renal mechanisms for the reabsorption of amino acids. One reabsorbs basic amino acids: lysine, arginine, ornithine, and cystine, a second reabsorbs glutamic and aspartic acids and a third reabsorbs un-ionized amino acids (Pitts, 1976). Experiments comparing the net tubular reabsorption of seventeen free amino acids indicated that while the percentage of amino acids reabsorbed was lower in infancy than childhood, when GFR was considered, the differences disappeared (Brodehl and Gellissen, 1968).

The accumulation of amino acids by renal cortical slices has been used to characterize the cellular mechanisms

for amino acid uptake in both mature and immature kidneys. Evidence indicates, however, that accumulation of amino acids by renal cortical slices reflects the uptake at the peritubular cell membrane (Foulkes, 1971). Uptake at the luminal membrane would reflect tubular reabsorption whereas uptake at the peritubular side probably has a nutritive role (Silbernagal et al., 1975). Despite the fact that uptake of amino acids by kidney slices does not represent renal reabsorption of amino acids, good correlations have been established repeatedly between the properties of amino acid reabsorption and the accumulation of amino acids by kidney slices. For example, L-lysine and L-ornithine inhibit both reabsorption of L-arginine from the tubular lumen (Bergeron and Morel, 1969) and its accumulation by slices (Rosenberg et al., 1962). These correlations justify the continued use of the slice technique to study amino acid transport systems of the kidney, particularly with immature kidneys where micropuncture studies are inappropriate.

Aminoisobutyric acid (AIB) is a non-metabolizable amino acid frequently used to study kidney slice uptake of amino acids. It is actively transported in the same way as metabolizable amino acids. Kidney slice accumulation of AIB is expressed as the ratio of the amount of AIB in the slices divided by the amount in the medium or the AIB S/M ratio.

Kidney slice accumulation of AIB has been determined in both immature and mature rats (Webber and Cairns, 1968; Segal et al., 1971), sheep (Scharrer et al., 1971), and humans (Solomon et al., 1976). In general, the rate of AIB uptake is slower in kidney slices from immature animals but the S/M ratios or the concentration gradients which can be achieved are greater (Webber and Cairns, 1968; Segal et al., 1971). This is partly explained by a slower rate of efflux of AIB from immature kidney slices (Webber, 1968). In rats, neonatal kidney slices have the highest AIB S/M ratio and these gradually decline to adult values by 15 days of age (Webber and Cairns, 1968; Segal et al., 1971). In humans this pattern of development occurs prenatally. The ability of kidney slices from seven to seventeen week old human fetuses to accumulate AIB decreased with increasing fetal age (Solomon et al., 1976). The characteristics of AIB accumulation by kidney slices from immature animals are different than those observed for adults. While in both, the uptake of AIB is sodium dependent and occurs as an electrogenic co-transport with sodium, only uptake by immature kidney slices can be enhanced by the addition of insulin to the incubation medium (Scharrer et al., 1971). Additionally, AIB uptake by kidney slices from immature animals is enhanced by preincubating slices in a Krebs-Ringer buffer for 30 minutes at 37^{o}C (Reynolds and Segal, 1976). This is not true for kidney slices from adults.

The enhanced uptake of AIB produced by preincubation is abolished by anaerobioses during the preincubation period. It is also abolished by the presence of cycloheximide or other protein synthesis inhibitors in the preincubation medium (Reynolds and Segal, 1976). Based on these findings, the authors suggested that a new protein, responsible for enhanced AIB transport, is synthesized during the preincubation period.

Secretion of Organic Ions

Active renal secretion of organic ions is another major proximal tubular function. Many substances are added to the urine by tubular secretion. Organic compounds such as sulfonic acids and carboxylic acids are secreted by a non-specific transport mechanism for organic acids. Compounds secreted by this transport system include phenol red, p-aminohippurate (PAH), penicillin, chlorothiazide, and glucuronides. A second mechanism transports a group of organic bases such as guanidine, thiamine, choline, histamine, tetraethylammonium (TEA) and n-methylnicotinamide (NMN) (Pitts, 1976). Secretion serves as a mechanism for excreting toxic metabolic products and foreign substances. Since most drugs are bound to plasma proteins and not filtered at the glomerulus, secretion is a major mechanism for drug excretion (Pitts, 1976). Of the two systems, the organic anion transport system has been most extensively

studied. This active transport system is relatively efficient. In man, the maximum secretory rate of PAH averages 80 mg/minute/1.73 m^2 of surface area (Pitts, 1976). The secretion of PAH by infants (8 days to 3 months of age) is approximately 30% below the secretion of PAH by adults (Weil, 1955).

Renal secretory mechanisms can be studied by the *in vitro* slice technique (Cross and Taggart, 1950). Active uptake of PAH by renal cortical slices reflects the *in vivo* tubular secretion of organic acids (Cross and Taggart, 1950). Similarly, the net accumulation of NMN or TEA by slices reflects the *in vivo* tubular secretion of organic bases (Farah and Rennick, 1956). Using the slice technique, the renal secretory capacity of immature rats (Kim *et al.*, 1972), dogs (Rennick, 1961; Hook *et al.*, 1970), and rabbits (Hirsch and Hook, 1970a,b) has been determined. In these species, PAH S/M ratios are low at birth and progressively increase with age. Peak values are reached from one to four weeks of age depending on the species, and thereafter decline to adult values. NMN S/M ratios are low at birth and gradually increase to adult values.

The organic anion secretory process can be induced. Pretreatment of pregnant or immature animals with organic acids such as penicillin causes a rapid development of this transport process in the fetus and the newborn.

Hirsch and Hook (1970) pretreated pregnant rabbits with penicillin during the last half of gestation. Kidneys from rabbits born of treated does developed increased PAH S/M ratios (Hirsch and Hook, 1969). Similarly, pretreatment of neonatal rats with penicillin enhanced PAH accumulation but did not alter NMN uptake (Kim et al., 1972). Pretreatment with penicillin also increased the clearance and transport maximum (Tm) of PAH in both infant rats (Noordewier and Withrow, 1976) and infant dogs (Bond et al., 1976) and the extraction of PAH in infant dogs (Bond et al., 1976) and infant rabbits (Kaplan and Lewy, 1978). Penicillin pretreatment had no effect on the secretory capacity of adult animals (Hirsch and Hook, 1970a). Since the administration of substrate to immature animals can stimulate the capacity to secrete organic acids, the stimulus to normal maturation may be an endogenous substrate load (Hirsch and Hook, 1970a).

Renal Excretion of Salt and Water Loads

The ability to excrete salt and water loads administered intragastrally or intravenously promptly and completely is characteristic in adult animals (Pitts, 1976). This ability is limited in the newborn of most species. In newborn rats, administration of a water load (5% of the body weight) does not produce a prompt water diuresis (Heller, 1947; Falk, 1955). Instead, increase in urine flow is

slight and delayed and there is little change in the osmotic pressure of the urine (McCance and Wilkinson, 1947). In 4-6 day old rats, water loading produces a small but significant water diuresis and a dilute urine (McCance and Wilkinson, 1947; Falk, 1955). In 10-12 day old rats, the response to a water load is not significantly different from that seen in adult rats (McCance and Wilkinson, 1947; Falk, 1955). In contrast, human infants do respond to a water load with a prompt water diuresis (McCance et al., 1954). However, the water diuresis produced in the human infant is less than that produced in the adult. Human infants and adults were given a water load (6% of body weight). After 4 hours, the adults excreted 90% of the load whereas the infants excreted only 50% of the load (McCance et al., 1954). As previously mentioned, the process of nephron formation is complete in the kidneys of newborn humans but not complete in the kidneys of newborn rats. Therefore, kidneys of newborn rats resemble those of fetuses of humans. This anatomical difference partly explains the more mature response to a water load observed in human infants when compared to rat infants, however, reasons for the smaller response to water loading by newborn compared to adult humans are not fully understood. The ability to excrete a water load depends on the amount of water presented to the ascending limb of the loop of Henle and the early convoluted tubule and the ability of

this segment to dilute the urine. The amount of urine delivered to the loop of Henle depends on GFR and proximal tubular water reabsorption (Loggie et al., 1975). GFR is lower in the kidneys of newborn animals of all species studied and therefore is partly responsible for the immature response to a water load. Since human newborn infants can dilute their urine to the same degree as adults (McCance et al., 1954) it is probable that the relatively low GFR observed in human infants is one major factor responsible for the immature response to a water load (Loggie et al., 1975).

Similarly, when an adult animal is given a large sodium load, the extracellular fluid (ECF) space immediately expands. The kidney responds by increasing GFR and decreasing the fractional sodium reabsorption in both the proximal and distal tubule (Davis et al., 1969). This results in a rapid excretion of sodium and return of ECF space to pre-load conditions. In contrast, when a newborn animal is given a large sodium load, serum sodium concentration increases, body weight increases, and a generalized edema develops (McCance and Widdowson, 1957). Administration of hypertonic saline solution to rats less than 3 weeks of age produced an osmotic diuresis that was smaller and delayed when compared to the adult response (McCance and Wilkinson, 1947; Falk, 1955). Similarly, newborn dogs excreted 5% of a sodium load administered intravenously

after 2 hours compared to 30% excretion by adult dogs given an equivalent load despite equivalent increases in GFR following the salt load (Kleinman and Hsieh, 1974). Factors that influence the relatively small renal response by immature animals to salt loading appear to be related to the distal rather than the proximal tubule. Kleinman and Hsieh (1974) reported that after administration of i.v. saline loads to newborn dogs, fractional sodium reabsorption was decreased in the proximal tubules but increased in the distal tubules. Since aldosterone is one factor that influences distal tubular sodium reabsorption, it could be partially responsible for the immature response. The concentration of aldosterone in the blood is higher in newborn infants than in adults (Siegel et al., 1974). This appears to be related in part to a lower excretory or metabolic breakdown rate of aldosterone in the newborn (Weldon et al., 1967).

Immature animals cannot concentrate the urine to the same degree as adults during dehydration (Heller, 1947; Falk, 1955). The ability to produce concentrated urine is primarily dependent on a high osmolar gradient between the medullary interstitium and cortical region. Kidneys of immature animals have lower osmolar gradients than those of adults (Stanier, 1972). As the animal matures, there is little change in the medullary sodium gradient but the urea gradient increases with age (Forrest and Stanier, 1966).

Infants fed a high protein diet or urea supplements concentrated their urine to a greater degree than infants on a low protein diet (Edelmann et al., 1960). Thus it appears that the maintenance of a urea gradient is a major factor in the ability to concentrate urine. A number of other factors may theoretically contribute to a low renal medullary osmolar gradient, however, more data are needed to determine their actual role.

Renal Regulation of Acid-Base Balance

The kidneys, the lungs and the blood buffers regulate the acid base balance in mammals. The role of the kidney involves both proximal and distal tubular hydrogen ion secretion and proximal tubular bicarbonate reabsorption. Hydrogen ion secretion is dependent on its concentration gradient across the luminal membrane. Renal tubular fluid is buffered by phosphates, ammonia, and other substances which associate with the proton to maintain a concentration gradient from tubular cells to luminal fluid (Pitts, 1976). Approximately 50% of the hydrogen ion is buffered by phosphates and salts of weak acids and 50% is buffered by ammonia (Pitts, 1976). The maximal gradient is reached when the urinary pH equals approximately 4.5 (Pitts, 1976). Therefore the ability of the immature kidney to secrete hydrogen ions depends on the following: 1) the capacity of proximal and distal tubules to secrete hydrogen ions,

2) the capacity of the proximal tubular cells to produce ammonia and 3) the availability of buffers in the tubular fluid. Studies with immature animals indicate the ability to cope with an acid-base imbalance is reduced during the neonatal period (McCance, 1961; Goldstein, 1970).

McCance (1961) administered ammonium chloride to human infants and adults to induce a metabolic acidosis. The infants produced less urinary titratable acid (T.A.) and less urinary ammonia than adults. Infants have lower levels of serum phosphate than adults. This may account for their lower T.A. excretion. McCance and Widdowson (1960) administered buffered neutral phosphate with ammonium chloride to human infants. This treatment tripled the amount of T.A. excreted by the infants. Edelmann et al. (1967) found that full term human infants and adults have the same capacity to secrete hydrogen ions. Thus the decreased capacity to cope with an acid-base imbalance observed in the human infant is probably due to the decreased availability of buffers in the tubular fluid or a decreased capacity to produce ammonia. Preterm human infants, however, secrete less protons than full term infants (Svenningsen, 1974).

Similarly the ability to respond to an acid-base imbalance is lower in infant than adult rats (Goldstein, 1970). Goldstein (1970) administered ammonium chloride to infant and adult rats. The adults responded with a rapid

increase in ammonia excretion, but did not significantly increase T.A. excretion. The response of the infant rat was significantly less. In rats, the normal adult response to acidosis develops between 14 and 21 days of age (Goldstein, 1970). This maturational process appears to depend on a supply of acid since chronic administration of ammonium chloride to ten day old rats enhanced the rate of both ammonia and T.A. excretion (Goldstein, 1970).

The rate of renal excretion of ammonia is determined by two factors: 1) the diffusion of preformed ammonia from the blood, through the tubular cells, and into the luminal fluid and 2) ammoniagenesis in the proximal tubular cells (Pitts, 1976). The diffusion of ammonia, which is lipid soluble and easily passes through membranes, is determined primarily by urinary pH. The lower the pH of the tubular fluid, the more ammonia will diffuse from the tubular cell. Renal ammonia is produced primarily in the mitochondria of proximal tubular cells. These cells contain a number of enzymes that could be involved in the production of ammonia from amino acids. These include transaminases, glutaminase, and glutamate dehydrogenase (GDH). Generally, ammonia is produced from glutamine; glutamine is hydrolyzed to glutamate and ammonia by glutaminase. Glutamate is then deaminated by GDH to α-ketoglutarate and ammonia, a reaction which requires the reduction of NAD^+ to NADH. However, a number of amino acids can be transaminated to glutamate and

consequently be used for ammonia production (Pitts, 1976). Experiments in mature dogs and rats indicate that under normal conditions 73% of the urinary ammonia is derived from plasma amino acids, 43% from glutamine and 30% from alanine, glycine, and glutamic acid. Arterial ammonia provides the remaining 27%. In the presence of acidosis there is an increased renal extraction of glutamine from the circulation (Pitts, 1976). Using radioactive-labeled tracers, Pitts (1976) found that the major fraction of glutamine was converted to α ketoglutarate and ammonia. Most of the ammonia was excreted to buffer the protons. Eighty percent of the α ketoglutarate went to carbon dioxide while twenty percent was converted to glucose (Pitts, 1976).

All of the following biochemical factors are thought to affect renal ammonia synthesis: 1) glutaminase concentration (Janicki and Goldstein, 1969), 2) glutamate concentration (Goldstein, 1966), 3) gluconeogenic capacity (Goldstein, 1966), and 4) mitochondrial NAD^+/NADH ratio (Preuss, 1968; Preuss, 1969).

In the rat, renal glutaminase concentration is low in the first two weeks of life and increases to adult levels between the second and third week of life (Benyajati and Goldstein, 1975). The activity of this enzyme is therefore associated with development of the ability to increase ammonia excretion in response to acidosis.

Renal gluconeogenesis may influence ammonia production by regulating the concentration of glutamate (Goldstein and Harley-DeWitt, 1973). A rate determining enzyme in the gluconeogenic pathway is phospho-enol-pyruvate carboxy-kinase (PEPCK). Since the activity of this enzyme increases during metabolic acidosis (Goodman et al., 1966; Alleyne and Scullard, 1969), an increased rate of renal gluconeo-genesis is associated with the increased rate of ammonia excretion observed in response to acidosis.

The mitochondrial NAD^+/NADH ratio is thought to control the direction of the GDH reaction, thereby regulating glu-tamate concentration (Preuss, 1968; 1969). The lower the ratio, the lower the conversion of glutamate to ammonia and αketoglutarate.

Previous studies using adult rats have shown that cortical slices from kidneys of acidotic animals utilized more glutamine and formed more ammonia, glucose, and glu-tamate than kidney slices from nonacidotic rats (Paglaira and Goodman, 1970; Roobol and Alleyne, 1974). Goldstein and Harley-DeWitt (1973) determined the gluconeogenic capacity of kidney cortical slices from 10, 12, 14, 16, and 21 day old rats. Renal gluconeogenic capacity was significantly lower in 10 day old rats than in adults, but rose to adult levels by 12 days of age. The effect of metabolic acidosis on in vitro renal gluconeogenesis was measured in 21 and 36 day old rats (Fraser and Alleyne,

1974). In all rats with acidosis, there was a significant increase in renal gluconeogenesis. The effect of metabolic acidosis on renal gluconeogenesis has not been determined in rats less than 21 days old. However, it has been estimated by the determination of the effect of medium pH on the capacity of renal cortical slices from 10, 12, 14, 16, and 21 day old rats to produce glucose (Goldstein and Harley-DeWitt, 1973). The response of gluconeogenesis to decreasing medium pH from 7.7 to 7.0 was 56% lower in 10 day old rats than in adults but increased to adult levels by 14 days of age. The NAD^+/NADH ratios were significantly lower in kidneys from 10-21 day old rats than in those from adults (Goldstein and Harley-DeWitt, 1973). This study indicates that both a low gluconeogenic capacity and a low NAD^+/NADH ratio may contribute to the decreased renal ammoniagenic capacity observed in rats less than 14 days old.

Nutrition and Kidney Development

Alterations in pre- and postnatal nutrition can influence the normal morphological and physiological development of the kidney. The degree of influence depends on the type and duration of the nutritional insult as well as the species involved. Many studies investigating the effects of nutrition on renal development have used rats. Since the rat kidney is relatively immature at birth, one can

extrapolate to other species only with great care.

Postnatal protein calorie over and under nutrition can be produced in young rats by adjusting litter size (Winick and Noble, 1966). A normal litter contains 8-12 pups. Winich and Noble (1966) found that rats raised in 18-pup litters in comparison to those from normal litters had a reduced kidney cell number; kidney cell size was unaffected. This decrease in cell number was thought to be permanent since it persisted at 133 days of age. Similarly, rats raised in 3-6 pup litters had an increase in kidney cell number; again, kidney cell size was not affected. To permanently alter kidney cell number, the postnatal nutritional modification must occur early in neonatal life since these authors found that post weaning malnutrition had no effect on kidney cell number (Winick and Noble, 1966).

In addition to kidney cell number, kidney function is affected by postnatal nutritional alterations. Bond and co-workers (1977) adjusted rat litters to 5, 10 and 20 pups and measured the accumulation of PAH and NMN by renal cortical from both 5 and 10 day old rats. These data indicate that renal secretion of organic acids and bases is significantly lower in pups reared in large litters when compared to pups reared in normal or small litters.

Experimentally, prenatal malnutrition is produced primarily by restricting an essential dietary component during pregnancy. Protein restricted diets fed to rats

during pregnancy caused a number of morphological and functional changes in the kidneys of the offspring (Hall and Zeman, 1968; Zeman, 1968; Allen and Zeman, 1971; 1973a; 1973b). These offspring had smaller kidneys, with less total DNA and less total protein, fewer and less well differentiated glomeruli, and fewer collecting ducts when compared to controls (Zeman, 1968). Functionally, protein deprived offspring had a reduction in GFR, a depressed ability to handle a water or osmotic load, and a depressed response to ADH when compared to age matched controls (Hall and Zeman, 1968; Allen and Zeman, 1971; 1973a; 1973b). Renal tubular transport functions, as measured by the accumulation of PAH, NMN, and AIB, by renal cortical slices, were not affected by prenatal protein restriction (Goldstein et al., 1979). However, young rats nursed by protein deprived dams showed a decrease in body weight and kidney weight. Additionally, accumulation of both PAH and NMN by kidney slices was depressed in rats nursed by protein deprived dams (Goldstein et al., 1979). Since maternal protein deprivation during lactation decreases the quantity of milk available for nursing (Venkatachalam and Ramanathan, 1964), this is, in effect, another model for protein-calorie malnutrition and the effects observed on kidney development in the progeny are similar to those observed in progeny raised in very large litters (Bond et al., 1977).

Thus far, studies on the effects of pre- and postnatal overnutrition on renal development are few. As previously mentioned, early postnatal overnutrition produced a permanent increase in renal cell number (Winick and Noble, 1966), enhanced tubular secretory capacity (Bond et al., 1977), and has been related to an increase in single nephron GFR (Solomon and Čapek, 1972). Prenatal overnutrition can be produced by a simple operation in the rat. One uterine horn is ligated before the female is bred. Rats manipulated in this way gave birth to a small number of heavier pups (Van Marthens et al., 1972). Utilizing this model, Gumbrecht and co-workers (1977) examined the effects of prenatal overnutrition on kidney composition and function at 20 days of gestation and one day after birth. Bodies and kidneys of pups from ligated dams weighed significantly more than those of age matched controls, but kidney function and composition were the same for the two groups. In contrast, Bond and co-workers (1977) observed that kidney slices from 5 and 10 day old rats from ligated dams accumulated significantly more PAH and NMN than kidney slices from controls. These data suggest that development of the renal secretory function was enhanced by prenatal overnutrition.

Infant of the Diabetic Mother (IDM)

The newborn infant of the diabetic mother (IDM) has a number of metabolic problems resulting from an alteration

in prenatal nutritional status. Throughout gestation, the fetus of the diabetic receives more glucose, amino acids, and ketones than does the fetus of a normal infant (Felig, 1977). The quantity of excess nutrients available to the fetus depends on the severity of the maternal diabetes. While a direct relationship between maternal blood glucose concentration and infant birth weight has not been established in humans (Persson, 1975), evidence from animal studies indicates that mild to moderate maternal diabetes produces neonates that are normal or large for gestational age while severe maternal diabetes produces infants that are small for gestational age (Kervran et al., 1978). Similarly, some human IDMs are small for gestational age (Persson, 1975; Farquhar, 1976). While these are more commonly born to women with diabetic complications, some mothers with apparently uncomplicated diabetes produce such infants (Farquhar, 1976).

Approximately 20-30% of IDMs are born large for gestational age (LGA) (Persson, 1975; Farquhar, 1976; Kitzmiller et al., 1978). These infants have a higher percent fat and glycogen but a lower percent extracellular and total water than infants of normal mothers (INM) of comparable maturity (Osler and Pederson, 1960; Cheek et al., 1961). This difference in body composition could not occur unless there was a simultaneous increase in fetal insulin secretion. In fact, evidence to date indicates that the

fetus and infant of the diabetic mother are hyperinsulinemic (Block et al., 1974).

The major clinical problems of the IDM are associated with hyperinsulinemia. In a recent study, 47% of IDMs developed hypoglycemia, while 36% were LGA. Although macrosomia was associated with hypoglycemia, a significant number of IDMs of average weight developed hypoglycemia (Kitzmiller et al., 1978). Furthermore, fetal hyperinsulinemia appears to alter the maturation of other organ systems. For example, approximately 8% of IDMs suffer from respiratory distress syndrome (RDS) (Kitzmiller et al., 1978). Excess insulin may depress plasma cortisol levels in the fetal circulation (Sosenko et al., 1979). Cortisol stimulates the synthesis of lecithin, the principle component of surfactant (Sosenko et al., 1979). A decrease in the synthesis of surfactant could be responsible for the increased incidence of RDS in IDMs.

Other forms of neonatal morbidity observed in IDMs include hypocalcemia (22%), hyperbilirubinemia (19%) and congenital anomalies (9%). Interestingly, these are problems commonly observed in premature infants and some investigators have suggested that IDMs are premature for their size (Farquhar, 1976).

Renal Function in the IDM

The chemical homeostasis in newborn IDMs is not normal. Calculations of the corrected bromide space by Cheek et al.

(1961) showed that IDMs have less extracellular fluid and less total body water than normal infants. Since sodium and chloride are major electrolytes in the extracellular fluid, it is not surprising that Fee and Weil (1963) found total body sodium and chloride low in IDMs in comparison to normal infants. IDMs tend to develop an uncompensated metabolic acidosis with decreased plasma pH, increased pCO_2 and some decreases in plasma protein, chloride, and total base (Lowery et al., 1954; Segal et al., 1957). In addition, intrapartum fetal distress was diagnosed in 25% of the diabetic women who had spontaneous or induced labor. This diagnosis was based partly on scalp pH below 7.25 (Kitzmiller et al., 1978).

Both normal and abnormal electrolyte excretion in IDMs have been observed. Osler (1960) and Cook et al. (1960) found that IDMs excrete proportionately more sodium, potassium, chloride and nitrogen than infants of comparable weight and slightly more than infants of comparable gestation. Osler and Pederson (1960) reported that the mean electrolyte excretion in IDMs was higher than what is considered as normal for gestational age. Stapleton (1956), however, found electrolyte excretion in IDMs the same as in normal infants. Abnormal electrolyte excretion may reflect abnormalities in body composition, endocrine function and/or kidney function.

IDMs have been reported to have low normal and high plasma potassium levels (Farquhar, 1976), raised serum phosphate levels (Zetterstrom and Arnhold, 1958; Farquhar, 1976), and low plasma calcium levels (Zetterstrom and Arnhold, 1958; Tsang et al., 1972; 1975; Kitzmiller et al., 1978).

Maturation and function of the renal glomerulus appears to be normal in fetuses and infants of diabetic mothers. GFR, estimated by thiosulphate clearance, was the same in IDMs and normal infants (Osler, 1960). Naeye (1975) examined the kidneys of fetuses from diabetic women and found no histological changes in the glomeruli. The increase in amniotic fluid creatinine concentration throughout gestation indicates a normal development of fetal glomeruli. This pattern was observed in amniotic fluid of pregnant diabetic women (Cassady et al., 1975).

Recent evidence indicates, however, that fetal renal tubules may not mature normally during a diabetic pregnancy. Sokol and Hall (1977) examined the disappearance of beta$_2$ microglobulin (B_2M) from the amniotic fluid of pregnant diabetic women. This protein is normally reabsorbed by fetal proximal tubules and amniotic fluid B_2M concentration normally decreases as the proximal tubules mature and can reabsorb this protein. In pregnant diabetics, the amniotic fluid B_2M did not decrease. This led the authors to suggest that the fetal renal tubules do not mature properly during

diabetic pregnancy.

Animal Models of the IDM

The two most frequently used diabetogenic agents are alloxan and streptozotocin (Rerup, 1970). Both drugs are cytotoxic to the beta cells of the pancreas. Both drugs have undesirable side effects, particularly relative to nephrotoxicity. Streptozotocin, however, is less nephrotoxic than alloxan (Rakieten et al., 1963) and acts by reducing the NAD^+/NADH content of the islet cells. This is followed by beta cell necrosis and diabetes. The changes can be prevented by the simultaneous administration of nicotinamide (Dulin and Wyse, 1969).

Experimental maternal diabetes has been induced in Rhesus monkeys (Mintz et al., 1972; Cheek et al., 1974), guinea pigs (Junod et al., 1967), and rats (Solomon, 1959; Pitkin et al., 1971; Szalay and Gaal, 1975; Aerts and Van Assche, 1977; Kervran et al., 1978).

Solomon (1959) induced diabetes midgestationally in rats with alloxan and measured body weight, number of stillborn, and percent moisture, protein and fat in the fetuses. Mildly diabetic rats delivered young with birth weights greater than those of controls; there was also an increased incidence of stillborn young. Severely diabetic rats could not maintain pregnancy and produced no progeny. The proportions of body moisture, protein, and fat observed in

fetuses of mildly diabetic rats were no different than those of control offspring. In these experiments, pregnant diabetic rats did not receive insulin.

Pitkin et al. (1971) induced diabetes in virgin rats with streptozotocin, bred them and maintained three groups: pregnant diabetics that received no insulin, pregnant diabetics that received a daily injection of insulin and a nondiabetic control group. Fetal and placental composition wereexamined on day 21 of pregnancy. Fetuses of untreated diabetic rats were larger and had significantly more fat, less water, and more DNA than those of controls. Fetuses of insulin treated diabetics were no different than those of control rats.

Aerts and Van Assche (1977) induced diabetes in rats on day one of gestation to study the endocrine pancreas of the fetuses. Rats were injected with 30, 40, or 50 mg/kg streptozotocin to produce "mild" and "severe" diabetes. Increased neonatal loss was observed in progeny of severely diabetic rats but birth weights of the progeny of severely and mildly diabetic rats were no different than those of control offspring. The endocrine pancreas of fetuses and newborn rats from mildly diabetic dams showed islet hypertrophy and beta cell hyperplasia. Beta cells of fetuses of severely diabetic dams were degranulated. In these experiments, pregnant diabetic rats did not receive insulin.

Kervran et al. (1978) induced diabetes with streptozotocin in rats prior to mating. Pregnant rats were divided into "severe" diabetics (blood glucose concentration above 300 mg/dl) and "mild" diabetics (blood glucose concentration ranging from 100 to 200 mg/dl). When compared to control fetuses, fetuses from severely diabetic dams showed a slight decrease in body weight, a decrease in pancreatic insulin stores and a decrease in plasma insulin concentration. In contrast, fetuses from mildly diabetic rats compared to those from controls showed no change in body weight, an increase in pancreatic insulin, and an increase in plasma insulin concentrations. In these experiments, pregnant diabetic rats did not receive insulin.

These data show that rats born from diabetic dams can be considered animal models of the IDM. Additionally, rats born of dams with mild diabetes represent a model for prenatal overnutrition. Since alterations in prenatal nutritional status have been shown to effect the structural and functional development of the kidneys in the progeny (Hall and Zeman, 1968; Allen and Zeman, 1971; 1973a; 1973b; Bond et al., 1977) it was of interest to determine the effects of maternal diabetes on the development of kidney function in the offspring. Therefore, the first series of experiments was designed to determine the effects of maternal diabetes on 1) kidney weight and composition in the offspring and 2) the development of kidney function in the offspring

in such a way as to eliminate extra-renal factors. The second series of experiments was designed to determine the effect of maternal diabetes on the ability of the progeny to excrete a water, an isotonic saline, or a hypertonic saline load.

METHODS

Rat Model of the IDM

Female Sprague-Dawley rats were obtained from Spartan Research Inc., Haslett, MI or Harlan Industries Inc., Indianapolis, IN on day one of their first gestation. Rats were randomly separated into two groups. Rats from one group were injected i.p. with streptozotocin (50 mg/kg) (courtesy of Dr. W.E. Dulin, Upjohn Co.) dissolved in 0.1 M citric acid (pH adjusted to 3.8-4.2). Rats from the remaining group were injected with 0.1 M citric acid solution (pH adjusted to 3.8-4.2) and were considered controls. Two to four days after injections, animals were placed in metabolism cages and urine samples were collected and analyzed for glucose (Tes-tape, Eli Lilly and Co.). Animals showing 0.1% glucose in their urine were considered diabetic. Diabetic animals were injected s.c. each afternoon between 4 and 8 PM with 2-4 units of NPH Iletin (isophane insulin suspension, Eli Lilly Co.). In the first series, diabetic animals received a set dose of 2 units of insulin per day throughout pregnancy. In the second series, the insulin dose was adjusted between 2 and 4 units per day according to urinary glucose concentration. Controls received

subcutaneous injections of saline. On the 18th day of gestation, blood samples were collected 24 hours after the daily insulin or saline injection. Approximately one ml of blood was collected from the orbitol sinus into a small testtube pretreated with sodium fluoride (to inhibit glycolysis) and heparin (to inhibit clot formation). This was centrifuged and the plasma was separated from the red cells. The plasma was frozen and later deproteinized and analyzed for glucose by an enzymatic colorimetric assay (Sigma Chemical Co.).

Animals were housed in a temperature controlled room with a 12-12 hour light-dark cycle. All animals received a standard rat chow (Wayne Lab Blox; 24% crude protein; 4.0% crude fat; 4.0% crude fiber; and 68% carbohydrate by weight) and water ad libitum. Rats were allowed to deliver normally. At birth, live pups from diabetic mothers (PDMs) and live pups from control mothers (PCMs) were weighed, adjusted to 10 per litter, and crossfostered to lactating controls. Lactating controls used as foster mothers delivered litters at the same time as diabetic mothers.

Experiments

Two series of experiments were completed. In the first series, kidney composition and function were evaluated in 1, 5, 10, and 28 day old PDMs and PCMs using *in vitro* kidney slice techniques. In the second series, ability of PDMs and

PCMs to excrete water and salt loads was determined using *in vivo* techniques.

Series I

Body Weight, Kidney Weight, and Kidney Cortex Composition

One, 5, 10, and 28 day old progeny were weighed and decapitated; the kidneys were removed, decapsulated, and weighed. Total water content of kidney cortex slices was determined as the difference between wet and dry weight. To obtain a dry weight, slices were placed in pre-weighed crucibles and dried at 100°C for 24 hours. Total protein content of renal cortical slices was determined by dissolving the slices in 3 N KOH, diluting each sample and assaying for protein according to the Lowry procedure (Lowry *et al.*, 1951).

In Vitro Renal Tubular Transport Capacity

Animals were killed by cervical dislocation. Kidneys were immediately removed, decapsulated, and placed in cold isotonic saline (0.9% sodium chloride).

The renal slice preparation described by Cross and Taggart (1950) was used. Renal cortical slices 0.2-0.3 mm thick were prepared freehand. The accumulation of the prototype organic acid, p-aminohippuric acid (PAH) and the prototype organic base, N-methylnicotinamide (NMN) by the slices was used as an index of renal intrinsic secretory

function. Approximately 50 mg of renal cortical slices was incubated in 2.7 mls of Cross and Taggart medium (1950) 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) and adjusted to pH 7.4. Incubations were carried out in a Dubnoff Metabolic Shaker at 25°C under 100% oxygen for 90 minutes (Cross and Taggart, 1950).

The accumulation of the non-metabolizable sugar, αmethyl glucoside (αMG) by renal cortical slices was used to determine the intrinsic renal transport capacity for sugars. Approximately 50 mg of kidney cortex slices was incubated in 3 ml of Krebs-Ringer Bicarbonate Buffer (Krebs et al., 1963) containing 2 mM αMG (Sigma Chemical Co.) and 0.2 μCi/ml ^{14}C αMG (New England Nuclear). Incubations were carried out in stoppered reaction flasks filled with 95% oxygen 5% carbon dioxide in a Dubnoff Metabolic Shaker at 37°C for 90 minutes (Segal et al., 1973a).

The accumulation of the non-metabolizable amino acid, aminoisobutyric acid (AIB) by renal cortical slices was used to determine the capacity of kidney slices to accumulate amino acids. Approximately 50 mg of tissue was preincubated in 3 ml of Kreb-Ringer Bicarbonate Buffer (Krebs et al., 1963). Preincubations were carried out in stoppered reaction flasks filled with 95% oxygen-5% carbon dioxide in a Dubnoff Metabolic Shaker at 37°C for 30 minutes. After preincubation, slices were transferred to a similar medium

containing 0.065 mM AIB (Sigma Chemical Co.) and 0.1 μCi/ml ^{14}C-AIB (New England Nuclear) and were incubated in stoppered reaction flasks filled with 95% oxygen-5% carbon dioxide in a Dubnoff Metabolic Shaker at 37°C for 60 minutes (Reynolds and Segal, 1976).

After incubation, slices were removed from the medium, blotted, weighed and placed in tissue homogenizers with 3 mls of trichloroacetic acid (TCA, 10%). Similarly, 2 mls of medium were mixed with 3 mls of TCA. Both medium and tissue homogenates were brought to a final volume of 10 mls with distilled water and centrifuged at 1400 rpm for 10 minutes. A 1.0 ml aliquot of supernatant was used to determine PAH spectrophotometrically as described by Smith et al. (1945). Additionally, a 1.0 ml aliquot was added to scintillation vials containing 10 mls of modified Bray's Solution (6 gm of 2,5-diphenyloxazole and 100 gm of naphthalene per liter of dioxane). Radioactivity of ^{14}C-NMN, ^{14}C-αMG, or ^{14}C-AIB in the supernatants was determined using a Beckman LS-250 liquid scintillation counter employing internal standards. Transport was expressed as the slice to medium (S/M) ratio. This was calculated as the concentration of PAH per gram of tissue (wet weight) divided by the concentration of PAH per ml of media. Similarly, in the case of ^{14}C-NMN, ^{14}C-αAIB, or ^{14}C αMG, disintegrations per minute (dpm) per gram of tissue (wet

weight) was divided by dpm per ml of medium.

In Vitro Renal Gluconeogenic and Ammoniagenic Capacity

Slices were prepared as previously described. The technique described by Krebs et al. (1963) was used to quantify renal gluconeogenic and ammoniagenic capacity. Approximately 20-40 mg of kidney cortex slices was incubated in 5 ml of Krebs-Ringer Bicarbonate Buffer both in the presence and absence of substrate, 10 mM sodium glutamate (Sigma Chemical Co.). Incubation flasks were filled with 95% oxygen- 5% carbon dioxide, stoppered, and incubated in a Dubnoff Metabolic Shaker at 37°C for 90 minutes. Slices were removed from the flasks, blotted and dried in pre-weighed crucibles at 100°C for 24 hours to determine dry weights. After removal of slices, 0.5 ml 10% (V/V) $HClO_4$ was mixed with the medium and the sample was centrifuged to precipitate protein. The supernatant was assayed for ammonia (Kaplan, 1965) and glucose (Glucostat, Worthington Biochemical Corp.). Net production of glucose or ammonia was calculated as the difference between glucose or ammonia produced in the presence of substrate and that produced in the absence of substrate. Results were expressed as micromoles of glucose or ammonia produced per hour per gram dry slice weight (μmol/hr/gm).

Response to Acidosis

Ten day old PDMs and PCMs were gavaged via polyethylene tube (PE-10) with ammonium chloride (Sigma Chemical Co.) (5 mM/kg; 2.5% of the body weight) or water (2.5% of the body weight. After 2.5-3.5 hours the rats were decapitated, the kidneys removed, decapsulated and weighed. *In vitro* renal gluconeogenic and ammoniagenic capacities were determined as previously described.

Series II

Animals

Pregnant control and diabetic rats were treated as previously described. Rats were allowed to deliver normally. At birth, PDMs and PCMs were weighed and transferred to lactating controls. Each lactating control received 5 PDMs and 5 PCMs. On the day of the experiment, 5 and 10 day old rats were taken from the dams and placed in beakers lined with cage bedding. Throughout the experiment, pups were warmed to 30-32^{o}C by a light bulb (McCance and Wilkinson, 1947).

Urine Collection

The bladder of each infant rat was emptied before each treatment at 2 hour intervals by gentle suprapubic pressure and stroking of the perineum. This method takes advantage of the fact that infant rats cannot urinate spontaneously, but instead urinate in response to a perineal stimulus

(McCance and Wilkinson, 1947). The urine was collected by a syringe and transferred to preweighed vials.

Salt and Water Loading

Five and ten day old PDMs and PCMs were removed from their mothers and placed in beakers under an electric light bulb. The ambient temperature was maintained at 30-32^{o}C. Each pup was weighed and the bladder was emptied. Each rat was gavaged via polyethylene tube (PE-10) with 5% of its body weight of one of the following solutions: distilled water, 0.15N sodium chloride (isotonic saline), or 0.5N sodium chloride (hypertonic saline). Animals in the fourth group received 5% of their body weight of distilled water and a s.c. injection of ADH (0.1mU/g; Pitressin, Parke Davis Co.). Two to four drops of green food color (U.S. Certified, Kroger) were added to 100 mls of each solution. This allowed easy detection of regurgitation or accidental esophogeal perforation. PCMs and PDMs receiving the same treatment were suckled by the sam dam. Bladders were emptied 2 and 4 hours after treatment. Urinary volume was determined by weighing the urine. Urinary sodium and potassium concentrations were determined with a flame photometer (Instrumentation Laboratory Inc.). Urinary chloride concentration was determined with a Buchler-Cotlove chloridometer (Buchler Instruments Inc.). Urinary nitrogen was determined colorimetrically with a modified urease Berthelot

reaction (Sigma Chemical Co.). Urinary osmolality was determined with a vapor pressure osmometer (Wescor, Inc.). Results are expressed in terms of concentration (mOsm, mM or mEq/liter) and excretion (mOsm, mM, or mEq/hour/weight).

Statistical Analysis

Series I

Except for the frequency distribution (Figures 1 and 2) all data were reported as means ± SEM. Differences between the means were analyzed using the Least Significant Difference (LSD) test after analysis of variance (completely random design). A paired-t-test was used to analyze the effect of acidosis on renal glucose and ammonia production between littermates (Table 3). The .05 level of probability was used as the criterion of significance (Steel and Torrie, 1960).

Series II

All data were reported as means ± SEM. Differences between the means were analyzed using the "Improved" Bonferroni-t-test (Games, 1977) after analysis of variance (factorial design and split plot design). The .05 level of probability was used as the criterion of significance (Steel and Torrie, 1960).

RESULTS

Maternal Plasma Glucose and Infant Birth Weight

Plasma glucose concentration was significantly greater in diabetic than control rats in both series of experiments (Table 1). The average birth weight of live pups from diabetic mothers (PDMs) (6.23 ± 0.17) was not significantly different than that of pups from control mothers (PCMs) (6.5 ± 0.11) from Series I (Table 1). In Series I, 55% of the PDMs had birth weights below the grand mean (the average birth weight for all rats: 6.34), while 33% of PCMs had birth weights below the grand mean (Figure 1). Similarly, the average birth weight of live PDMs (7.12 ± 0.21) was no different than that of PCMs (6.85 ± 0.15) from Series II (Table 1). In Series II, when litters included at least 5 pups, 78% of PDMs had birth weights greater than the grand mean (6.98) while only 42% of PCMs had birth weights greater than the grand mean (Figure 2; Top). In addition, to correct for small litter size, a frequency distribution of average birth weights for litters containing 9 to 12 pups is shown on the bottom of Figure 2. Seventy-one percent of the PDMs had birth weights above the grand mean while only 22% of the PCMs had birth weights above the grand mean (Figure 2;

Table 1. The relationship between maternal plasma glucose concentration and birth weight of the progeny of diabetic and control Sprague-Dawley rats

	Maternal Plasma Glucose[a] (mg/dl)	Birth Weight[b] (g)	Correlation Coefficient (r)
Series I			
Control	96.7 ± 4[c](22)	6.50 ± 0.11(22)	-0.192(44)
Diabetic	321.9 ± 33(22)*	6.23 ± 0.17(22)	
Series II			
Control	97.1 ± 5(10)	6.85 ± 0.15(8)	0.220(18)
Diabetic	435.4 ± 22(11)*	7.12 ± 0.21(10)	

[a]Blood samples were collected on the 18th day of gestation from the orbital sinus 24 hours after the daily insulin or saline injection.

[b]Birth weight of live pups was determined by dividing litter weight by the number of pups.

[c]Each value represents the mean ± SEM (number of litters).

*Signicicantly different from control (student's t; $p<.05$).

Figure 1. The effect of gestational diabetes on the birth weight of the progeny from Series I. Each bar represents the number of litters with the average pup weight falling in the indicated range. All litters contained at least 8 pups.

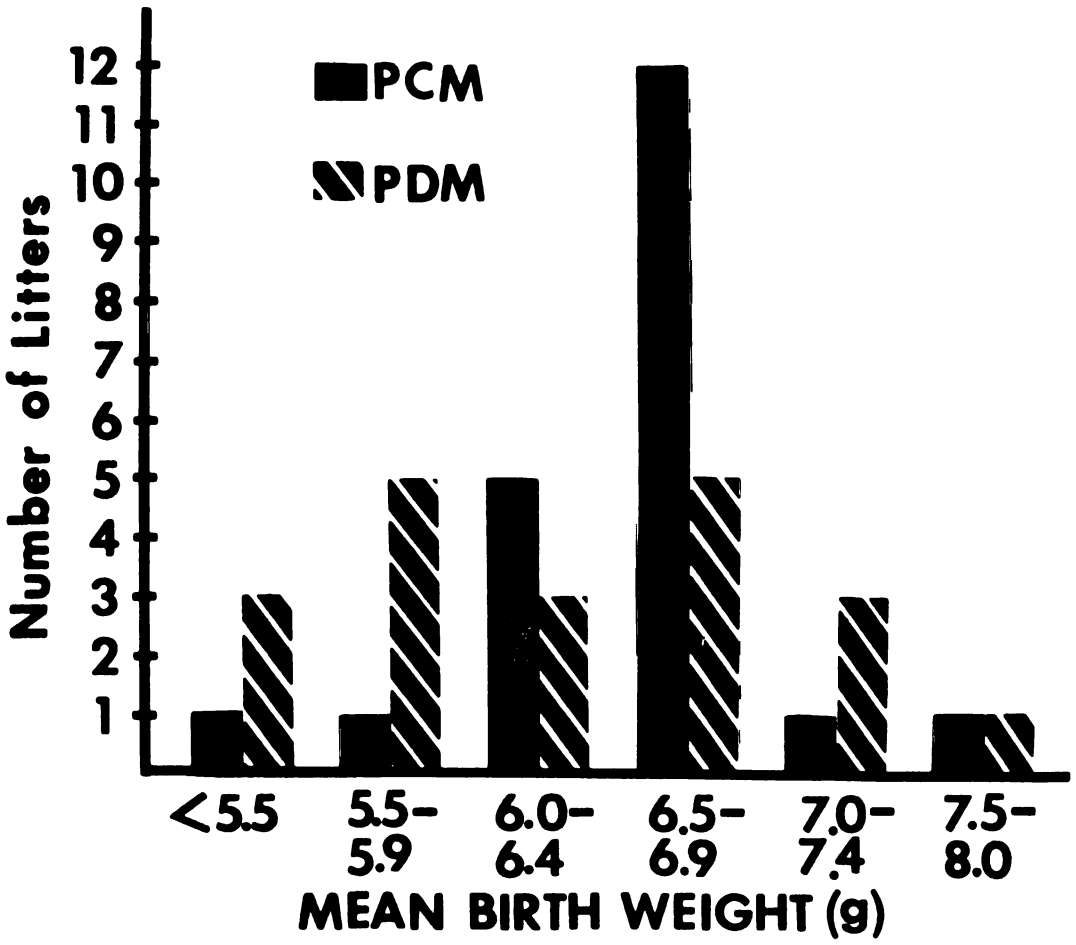
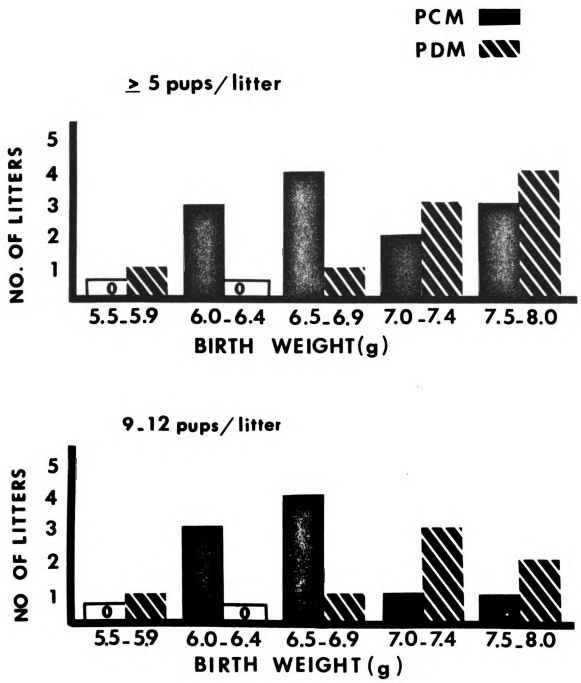

PCM
PDM
Number of Litters
12
11
10
9
8
7
6
5
4
3
2
1
<5.5
5.5–5.9
6.0–6.4
6.5–6.9
7.0–7.4
7.5–8.0
MEAN BIRTH WEIGHT (g)

Figure 2. The effect of gestational diabetes on the birth weight of the progeny from Series II. Each bar represents the number of litters with the average pup weight falling in the indicated range.

Top: Each litter contained at least 5 pups.

Bottom: Each litter contained 9 to 12 pups.


PCM
PDM
≥ 5 pups/litter
NO. OF LITTERS
5
4
3
2
1
0
0
5.5-5.9
6.0-6.4
6.5-6.9
7.0-7.4
7.5-8.0
BIRTH WEIGHT(g)
9-12 pups/litter
NO OF LITTERS
5
4
3
2
1
0
0
5.5-5.9
6.0-6.4
6.5-6.9
7.0-7.4
7.5-8.0
BIRTH WEIGHT(g)

Bottom).

Fetal and Neonatal Mortality

The number of fetal and neonatal deaths is an indicator of the severity of maternal diabetes (Solomon, 1959). In the first series of experiments, 13% of the PDMs were born dead and 7.4% died within 24 hours after birth (Table 2). These rates were significantly greater than those for controls in the same series of experiments (1.5% and 0% respectively) (Table 2). In the second series of experiments, however, the stillbirth rate and neonatal death rate were comparable in the two groups (Table 2).

Body Weight and Kidney Weight

Body weight and kidney weight of PDMs were no different than those of PCMs at 1, 5, or 10 days of age (Figures 3 and 4). However, by 28 days of age, PDMs weighed 29% less than PCMs (66 ± 6 g verses 83 ± 2 g). Similarly, the kidneys from PDMs weighed 20% less than those from PCMs (0.63 ± 0.05 verses 0.78 ± 0.40). The kidney weight/body weight ratios for PDMs were no different than those for PCMs at any age studied.

Percent of Water and Protein in the Renal Cortex

The percentage of water (Figure 5) and protein (Figure 6) in renal cortical slices from PDMs was no different than that of PCMs at any age.

Table 2. Effect of gestational diabetes on fetal and neonatal mortality

	Series I		Series II	
	Control	Diabetic	Control	Diabetic
Number of litters	22	18	10	10
Number of young (average per litter)	11.7	11.9	7.9	9.8
Total number of young	263	215	83	98
Live born young	259	187	79	92
Still born young[a]	4	28	4	6
Still born rate (%)	1.52	13*	4.8	6.1
Neonatal deaths[b]	0	16	0	0
Neonatal death rate (%)	0	7.4*	0	0

[a]Does not include loss of entire litters.

[b]Neonatal deaths include those rats that were alive at birth but died within 24 hours.

[c]Significantly different from control ($(Chi)^2$ test; $p<.05$).

Figure 3. Effect of gestational diabetes on kidney weight of the progeny. Each bar represents the mean ± SEM obtained from at least 5 observations. One observation consisted of kidneys pooled from one liter. Asterisks denote a statistical difference between PCM and PDM within an age group ($p<.05$).

Figure 4. Effect of gestational diabetes on body weight of the progeny. Each bar represents the mean ± SEM obtained from at least 5 observations. One observation consisted of kidneys pooled from one liter. Asterisks denote a statistical difference between PCM and PDM within an age group ($p<.05$).

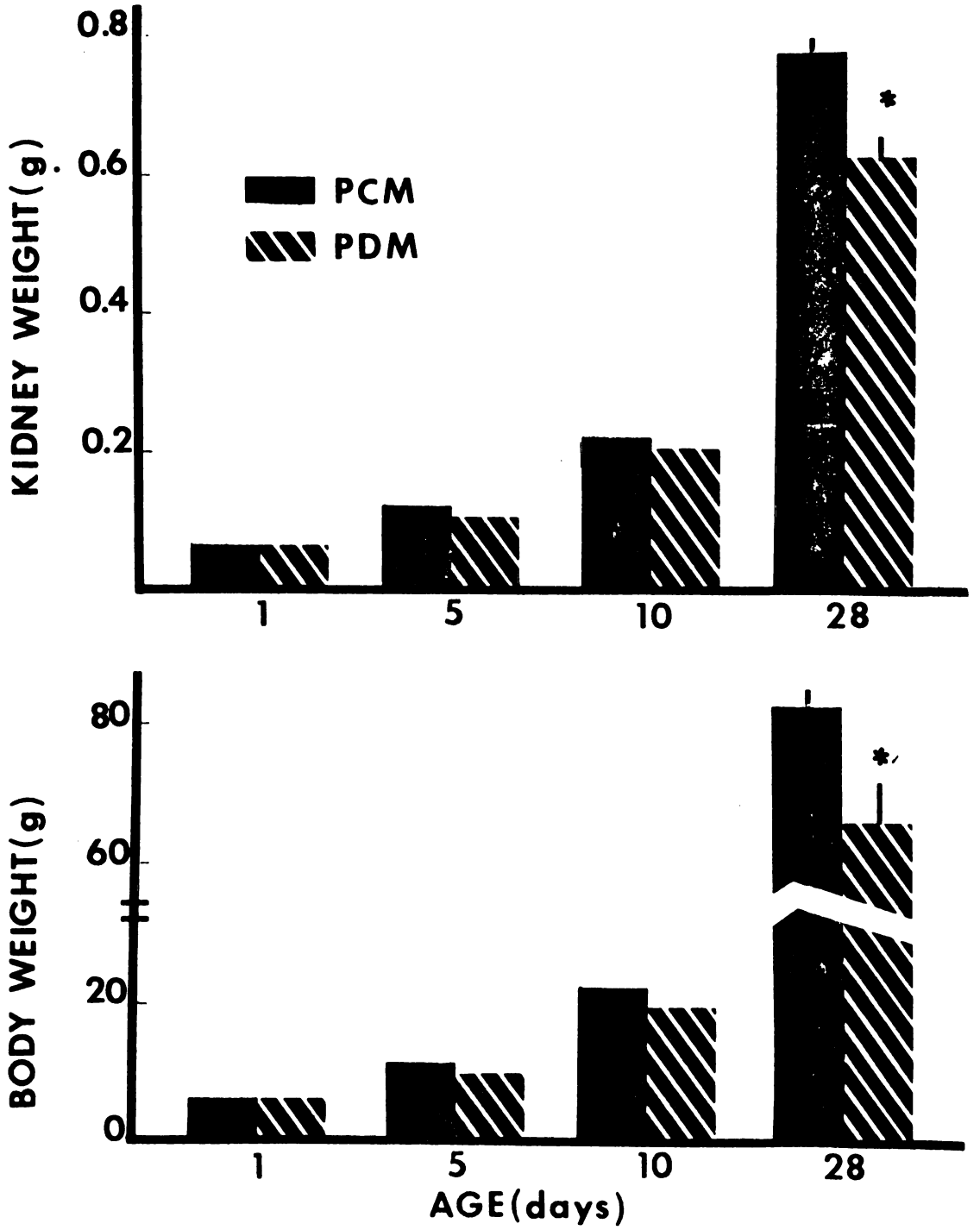
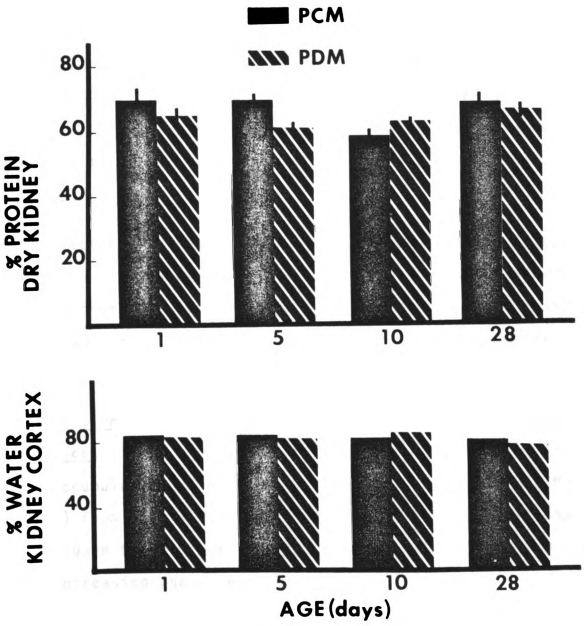

KIDNEY WEIGHT(g)
0.8
0.6
0.4
0.2
PCM
PDM
*
1
5
10
28
BODY WEIGHT(g)
80
60
20
0
*
1
5
10
28
AGE(days)

Figure 5. Effect of gestational diabetes on the percent of protein of cortical slices from kidneys of the offspring. Each bar represents the mean ± SEM obtained from at least 5 observations. One observation consisted of pooled slices from one liter.

Figure 6. Effect of gestational diabetes on the percent of water of cortical slices from kidneys of the offspring. Each bar represents the mean obtained from at least 5 observations. One observation considted of pooled slices from one liter. The SEM was too small to be shown with the bars.


PCM
PDM
% PROTEIN DRY KIDNEY
80
60
40
20
1
5
10
28
% WATER KIDNEY CORTEX
80
40
1
5
10
28
AGE (days)

Accumulation of Organic Acid (PAH) by Renal Cortical Slices

Accumulation of PAH by renal cortical slices (PAH S/M ratios) increased gradually from 1 to 28 days of age in PCMs. In PDMs, PAH S/M ratios increased from 1 to 5 days of age, decreased at 10 days of age, and increased to control values by 28 days of age (Figure 7). PAH S/M ratios of PDMs were no different than those of PCMs at 1, 5, or 28 days of age. However, at 10 days of age, PAH S/M ratios of PDMs were 32% lower than those of PCMs (6.41 ± 0.8 verses 9.75 ± 1.4) (Figure 7). This difference was statistically different.

Accumulation of Organic Base (NMN) by Renal Cortical Slices

Accumulation of NMN by renal cortical slices (NMN S/M ratios) from PDMs was no different from that of PCMs at any age (Figure 8). In both groups, NMN S/M ratios gradually increased from 1 to 28 days of age (Figure 8).

Accumulation of αAminoisobutyric Acid (AIB) by Renal Cortical Slices

Accumulation of AIB by renal cortical slices (AIB S/M ratios) from PDMs was no different than that of PCMs at any age (Figure 9). In both groups, AIB S/M ratios decreased with increasing age (Figure 9).

Figure 7. Effect of gestational diabetes on PAH accumulation by cortical slices from kidneys of the progeny (PAH S/M ratio). Each bar represents the mean ± SEM of at least 6 determinations. Asterisks denote a statistical difference between PCM and PDM within an age group ($p<.05$).

Figure 8. Effect of gestational diabetes on NMN accumulation by cortical slices from kidneys of the progeny (NMN S/M ratio). Each bar represents the mean ± SEM of at least 6 determinations.

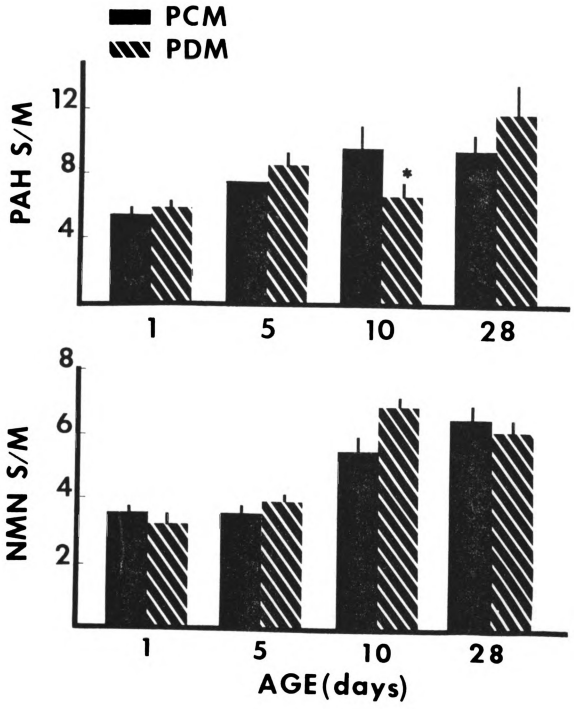
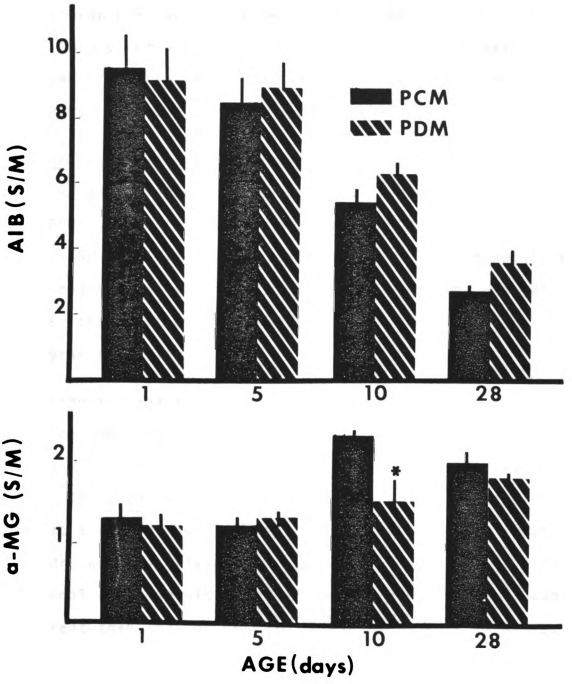

PCM
PDM
PAH S/M
12
8
4
*
1
5
10
28
NMN S/M
8
6
4
2
1
5
10
28
AGE(days)

Figure 9. Effect of gestational diabetes on AIB accumulation by cortical slices from kidneys of the progeny (AIB S/M ratio). Each bar represents the mean ± SEM of at least 7 determinations.

Figure 10. Effect of gestational diabetes on αMG accumulation by cortical slices from kidneys of the progeny (αMG S/M ratio). Each bar represents the mean ± SEM of 4 determinations. Asterisks denote a statistical difference between PCM and PDM within an age group ($p<.05$).


AIB (S/M)
10
8
6
4
2
PCM
PDM
1
5
10
28
a-MG (S/M)
2
1
*
1
5
10
28
AGE (days)

Accumulation of αMethylglucoside (αMG) by Renal Cortical Slices

Accumulation of αMG by renal cortical slices (αMG S/M ratio) from PDMs was no different from that of PCMs at 1, 5, or 28 days of age (Figure 10). However, at 10 days of age, αMG S/M ratios from PDMs were 31% lower than those from PCMs (1.58 ± 0.1 verses 2.3 ± 0.3) (Figure 10).

Gluconeogenic Capacity of Renal Cortical Slices

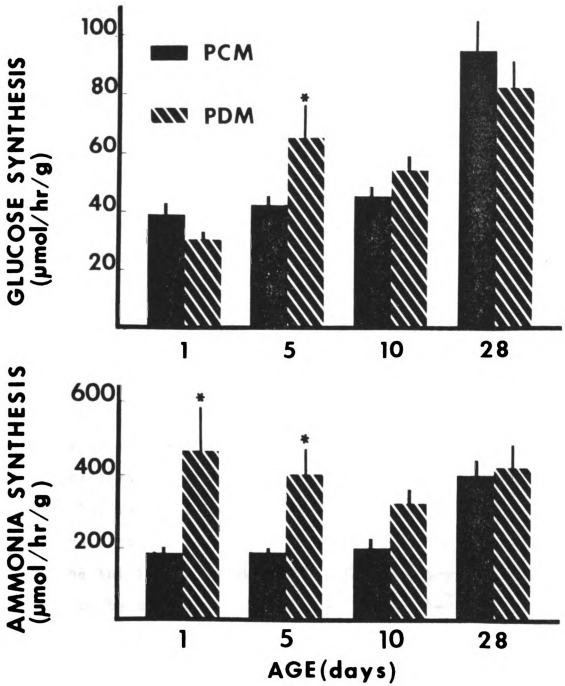
The gluconeogenic capacity of renal cortical slices from PDMs was no different than that of PCMs at 1, 10, or 28 days of age (Figure 11). However, at 5 days of age, the gluconeogenic capacity of kidney slices from PDMs was 50% greater than that of PCMs (64 ± 11 verses 43 ± 3.4 μmol/hour/gram dry weight).

Ammoniagenic Capacity of Renal Cortical Slices

The ammoniagenic capacity of renal cortical slices from PDMs was 300% that of PCMs at one day of age (456 ± 130 versus 182 ± 33 μmol/hour/gram dry weight) and 200% that of PCMs at 5 days of age (380 ± 72 verses 187 ± 24 μmol/hour/gram dry weight) (Figure 12). Although this trend persisted, the renal ammoniagenic capacity of PDMs was not significantly different than that of PCMs at 10 days of age. Similarly, at 28 days of age, renal ammoniagenic capacity of PDMs was no different than that of PCMs (Figure 12).

Figure 11. Effect of gestational diabetes on gluconeogenic capacity of kidney cortex slices from the progeny. Each bar represents the mean ± SEM of at least 7 determinations. Asterisks denote a statistical difference between PCM and PDM within an age group ($p<.05$).

Figure 12. Effect of gestational diabetes on ammoniagenic capacity of kidney cortex slices from the progeny. Each bar represents the mean ± SEM of at least 7 determinations. Asterisks denote a statistical difference between PCM and PDM within an age group ($p<.05$).


GLUCOSE SYNTHESIS
(μmol/hr/g)
100
80
60
40
20
PCM
PDM
*
1
5
10
28
AMMONIA SYNTHESIS
(μmol/hr/g)
600
400
200
*
*
1
5
10
28
AGE(days)

Effect of Acidosis on Glucose and Ammonia Production by Renal Cortical Slices

Kidney slices from 10 day old PDMs and PCMs treated with ammonium chloride (5mM/kg) produced slightly more glucose but no more ammonia than those from controls (Table 3). Renal gluconeogenic and ammoniagenic capacities of kidney slices from PDMs was no different than that of kidney slices from PCMs both in the presence and absence of acidosis (Table 3).

Effect of Gestational Diabetes on the Excretion of Water, Isotonic and Hypertonic Saline Loads by the Progeny

In general, PDMs and PCMs demonstrated a similar ability to compensate for water and isotonic saline loads (Figures 13 to 24; Tables 4 and 5). The urine, total solute, sodium, potassium, chloride and urea excreted in 4 hours relative to body weight was the same in 5 or 10 day old PDMs treated with isotonic saline or water when compared to age matched PCMs receiving the same treatment (Figures 13, 15, 17, 19, 23). While the ability to compensate for water and isotonic saline loads was similar in PDMs and PCMs, the ability to compensate for a hypertonic saline load appears to be different between the two groups. After a hypertonic saline load, total solute excretion by both 5 and 10 day old PDMs was significantly greater than that by age matched PCMs (Figure 15). Additionally, 10 day old PDMs

Table 3. Effect of acidosis on glucose and ammonia production by renal cortical slices from ten day old PDMs and PCMs

	Water[a]	NH_4CL[b]	Percent change
PCM			
Glucose (μmol/hr/g[c])	67.2 ± 1.9[d]	72.8 ± 2.0*	8.4
Ammonia (μmol/hr/g)	438 ± 18	403 ± 31	-
PDM			
Glucose (μmol/hr/g)	67.1 ± 6.6	71.8 ± 7.3*	6.3
Ammonia (μmol/hr/g)	406 ± 32	395 ± 35	-

[a]Rats were gavaged with water (2.5% of body weight).

[b]Rats were gavaged with ammonium chloride (5mM/kg; 2.5% of body weight).

[c]Dry weight.

[d]Each value represents the mean ± SEM obtained from 4 rats.

*Significantly different from control ($p<.05$).

Figure 13. Effect of gestational diabetes on the excretion of urine by the progeny during the first 4 hours after salt and water loading. Rats were gavaged with water, isotonic saline (0.15N sodium chloride) or hypertonic saline (0.5N sodium chloride) at 5% of the body weight. A fourth group received the same dose of water and a subcutaneous injection of ADH (0.1 mU/g). Bladders were emptied 2 and 4 hours after treatment. Each bar represents the mean ± SEM obtained from an "n" of 4. For 5 day old animals, urine from 2-5 rats was pooled for an "n" of 1. For 10 day old animals, urine from one rat was used for an "n" of 1. Means not sharing a common superscript differ significantly ($p<.05$).

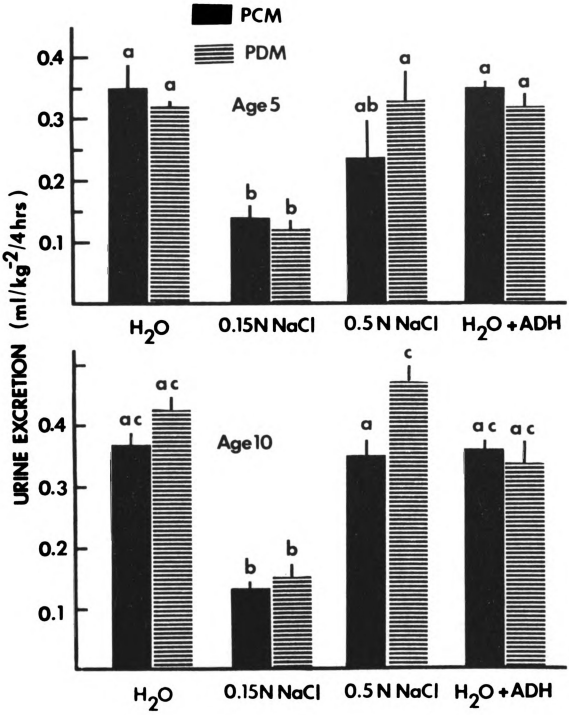

PCM
PDM
Age 5
Age 10
URINE EXCRETION (ml/kg-2/4hrs)
0.4
0.3
0.2
0.1
a
a
b
b
ab
a
a
a
ac
ac
b
b
a
c
ac
ac
H2O
0.15N NaCl
0.5 N NaCl
H2O +ADH

Figure 14. Effect of gestational diabetes on the excretion of urine by the progeny during the 0-2 and 2-4 hour periods after salt and water loading. Rats were gavaged with water, isotonic saline (0.15N sodium chloride) or hypertonic saline (0.5N sodium chloride) at 5% of the body weight. A fourth group received the same dose of water and a subcutaneous injection of ADH (0.1 mU/g). Bladders were emptied 2 and 4 hours after treatment. Each bar represents the mean ± SEM obtained from an "n" of 4. For 5 day old animals, urine from 2-5 rats was pooled for an "n" of 1. For 10 day old animals, urine from 1 rat was used for an "n" of 1. Means not sharing a common superscript differ significantly ($p<.05$).

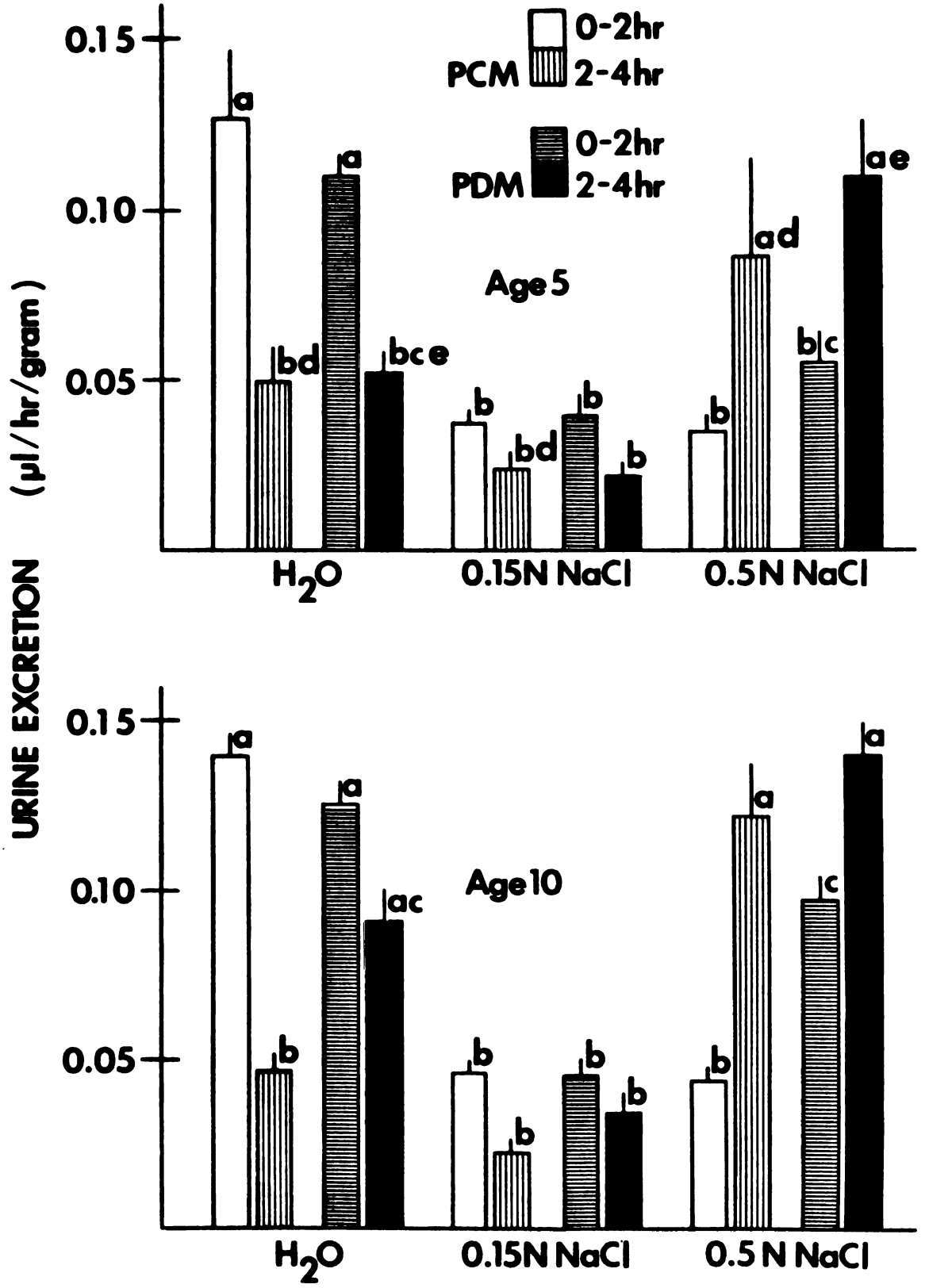

URINE EXCRETION (µl/hr/gram)
0.15
0.10
0.05
PCM 0-2hr
2-4hr
PDM 0-2hr
2-4hr
Age 5
a
bd
a
bce
b
bd
b
b
b
ad
bc
ae
H2O
0.15N NaCl
0.5N NaCl
Age 10
a
b
a
ac
b
b
b
b
b
a
c
a
H2O
0.15N NaCl
0.5 N NaCl

Figure 15. Effect of gestational diabetes on the excretion of total solute by the progeny during the first 4 hours after salt and water loading. Rats were gavaged with water, isotonic saline (0.15N sodium chloride) or hypertonic saline (0.5N sodium chloride) at 5% of the body weight. A fourth group received the same dose of water and a subcutaneous injection of ADH (0.1 mU/g). Bladders were emptied 2 and 4 hours after treatment. Each bar represents the mean ± SEM obtained from an "n" of 4. For 5 day old animals, urine from 2-5 rats was pooled for an "n" of 1. For 10 day old animals, urine from 1 rat was used for an "n" of 1. Means not sharing a common superscript differ significantly ($p<.05$).

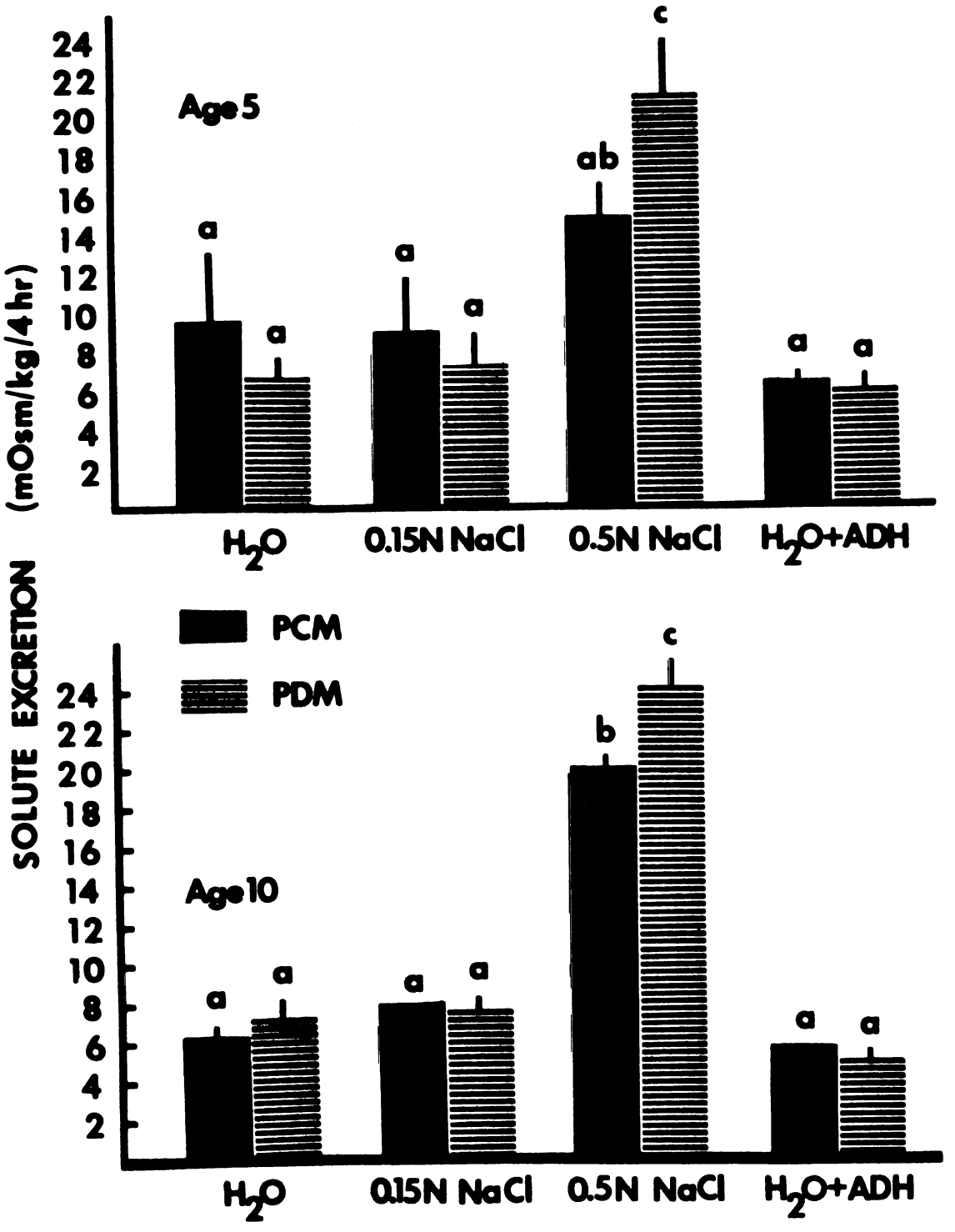

SOLUTE EXCRETION (mOsm/kg/4 hr)
Age 5
Age 10
PCM
PDM
24
22
20
18
16
14
12
10
8
6
4
2
a
a
a
a
ab
c
a
a
a
a
b
c
a
a
H2O
0.15N NaCl
0.5N NaCl
H2O+ADH

Figure 16. Effect of gestational diabetes on the excretion of total solute by the progeny during the 0-2 and 2-4 hour periods after salt and water loading. Rats were gavaged with water, isotonic saline (0.15n sodium chloride) or hypertonic saline (0.5n sodium chloride) at 5% of the body weight. A fourth group received the same dose of water and a subcutaneous injection of ADH (0.1 mU/g). Bladders were emptied 2 and 4 hours after treatment. Each bar represents the mean ± SEM obtained from an "n" of 4. For 5 day old animals, urine from 2-5 rats was pooled for an "n" of 1. For 10 day old animals, urine from 1 rat was used for an "n" of 1. Means not sharing a common superscript differ significantly ($p<.05$).

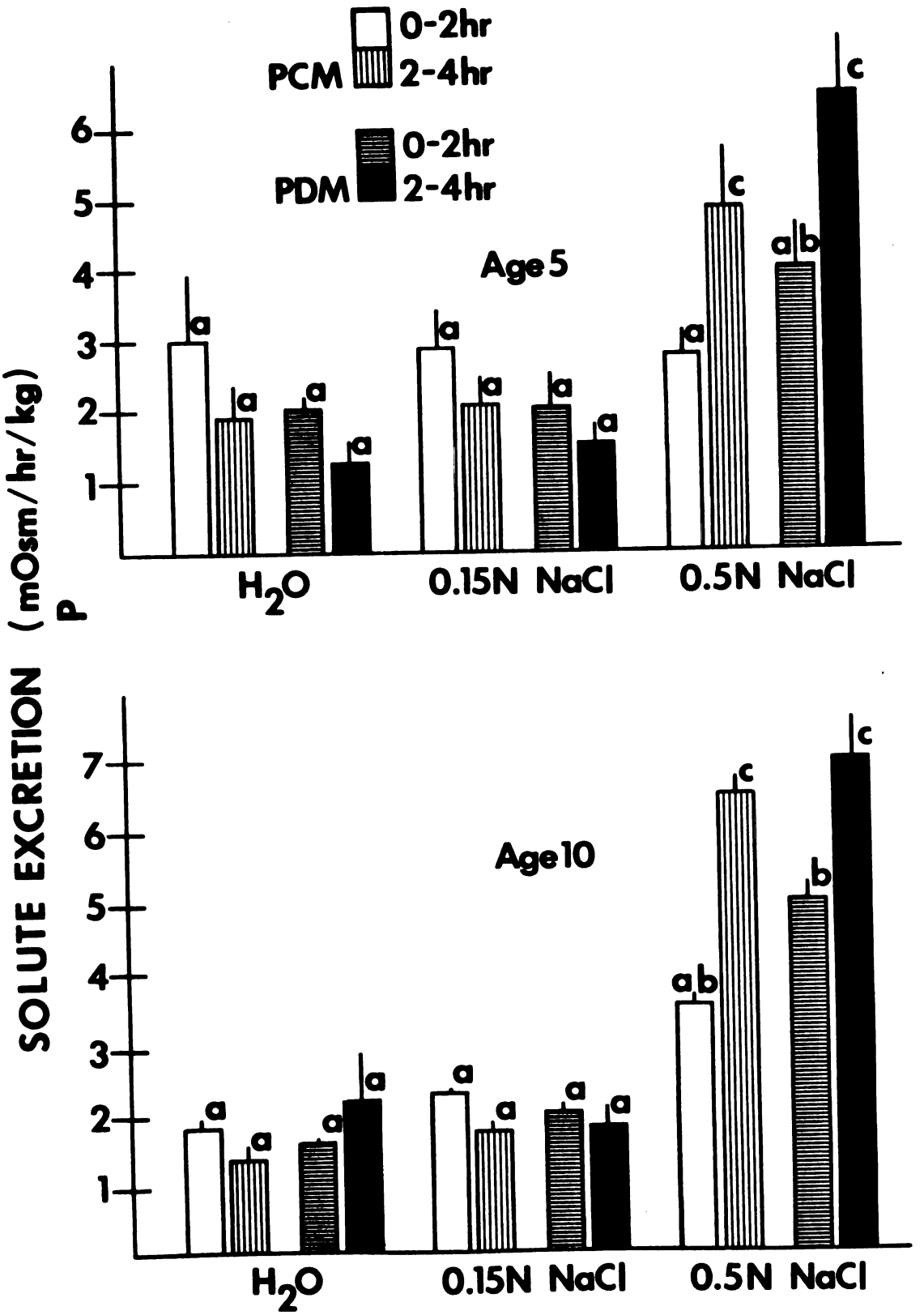

0-2hr
PCM
2-4hr
0-2hr
PDM
2-4hr
Age 5
Age 10
SOLUTE EXCRETION (mOsm/hr/kg)
P
H2O
0.15N NaCl
0.5N NaCl

Figure 17. Effect of gestational diabetes on the excretion of sodium by the progeny during the first 4 hours after salt and water loading. Rats were gavaged with water, isotonic saline (0.15N sodium chloride) or hypertonic saline (0.5N sodium chloride) at 5% of the body weight. A fourth group received the same dose of water and a subcutaneous injection of ADH (0.1 mU/g). Bladders were emptied 2 and 4 hours after the treatment. Each bar represents the mean ± SEM obtained from an "n" of 4. For 5 day old animals, urine from 2-5 rats was pooled for an "n" of 1. For 10 day old animals, urine from 1 rat was used for an "n" of 1. Means not sharing a common superscript differ significantly ($p<.05$).

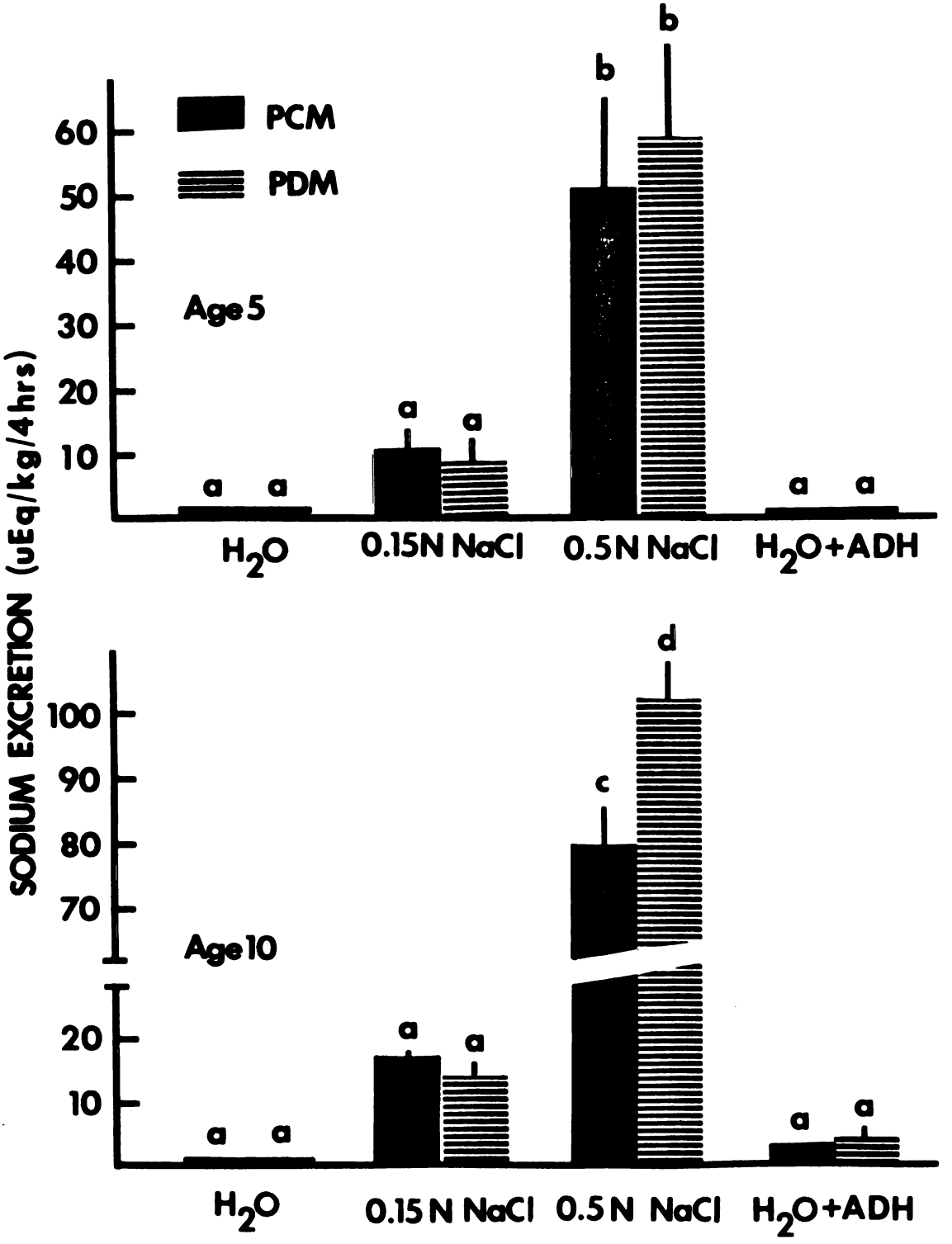

PCM
PDM
Age 5
Age 10
SODIUM EXCRETION (uEq/kg/4hrs)
60
50
40
30
20
10
100
90
80
70
a
b
c
d
H2O
0.15N NaCl
0.5N NaCl
H2O+ADH

Figure 18. Effect of gestational diabetes on the excretion of sodium by the progeny during the 0-2 and 2-4 hour periods after salt and water loading. Rats were gavaged with water, isotonic saline (0.15N sodium chloride) or hypertonic saline (0.5N sodium chloride) at 5% of the body weight. A fourth group received the same dose of water and a subcutaneous injection of ADH (0.1 mU/g). Bladders were emptied 2 and 4 hours after treatment. Each bar represents the mean ± SEM obtained from an "n" of 4. For 5 day old animals, urine from 2-5 rats was pooled for an "n" of 1. For 10 day old animals, urine from 1 rat was used for an "n" of 1. Means not sharing a common superscript differ significantly ($p<.05$).

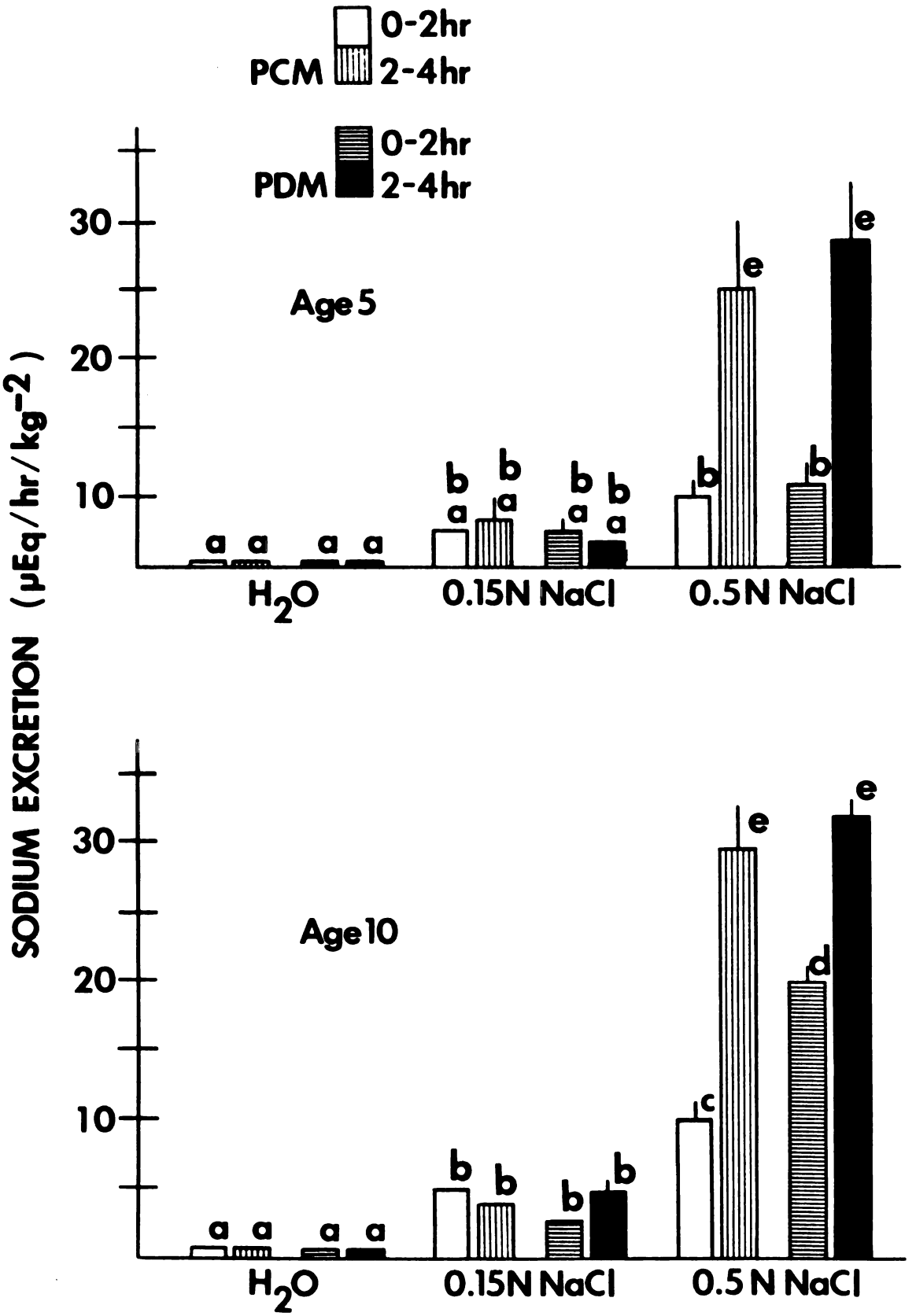

PCM
0-2hr
2-4hr
PDM
0-2hr
2-4hr
Age 5
Age 10
SODIUM EXCRETION (μEq/hr/kg⁻²)
30
20
10
H2O
0.15N NaCl
0.5N NaCl

Figure 19. Effect of gestational diabetes on the excretion of chloride by the progeny during the first 4 hours after salt and water loading. Rats were gavaged with water, isotonic saline (0.15N sodium chloride) or hypertonic saline (0.5N sodium chloride) at 5% of the body weight. A fourth group received the same dose of water and a subcutaneous injection of ADH (0.1 mU/g). Bladders were emptied 2 and 4 hours after treatment. Each bar represents the mean ± SEM obtained from an "n" of 4. For 5 day old animals, urine from 2-5 rats was pooled for an "n" of 1. For 10 day old animals, urine from 1 rat was used for an "n" of 1. Means not sharing a common superscript differ significantly ($p<.05$).

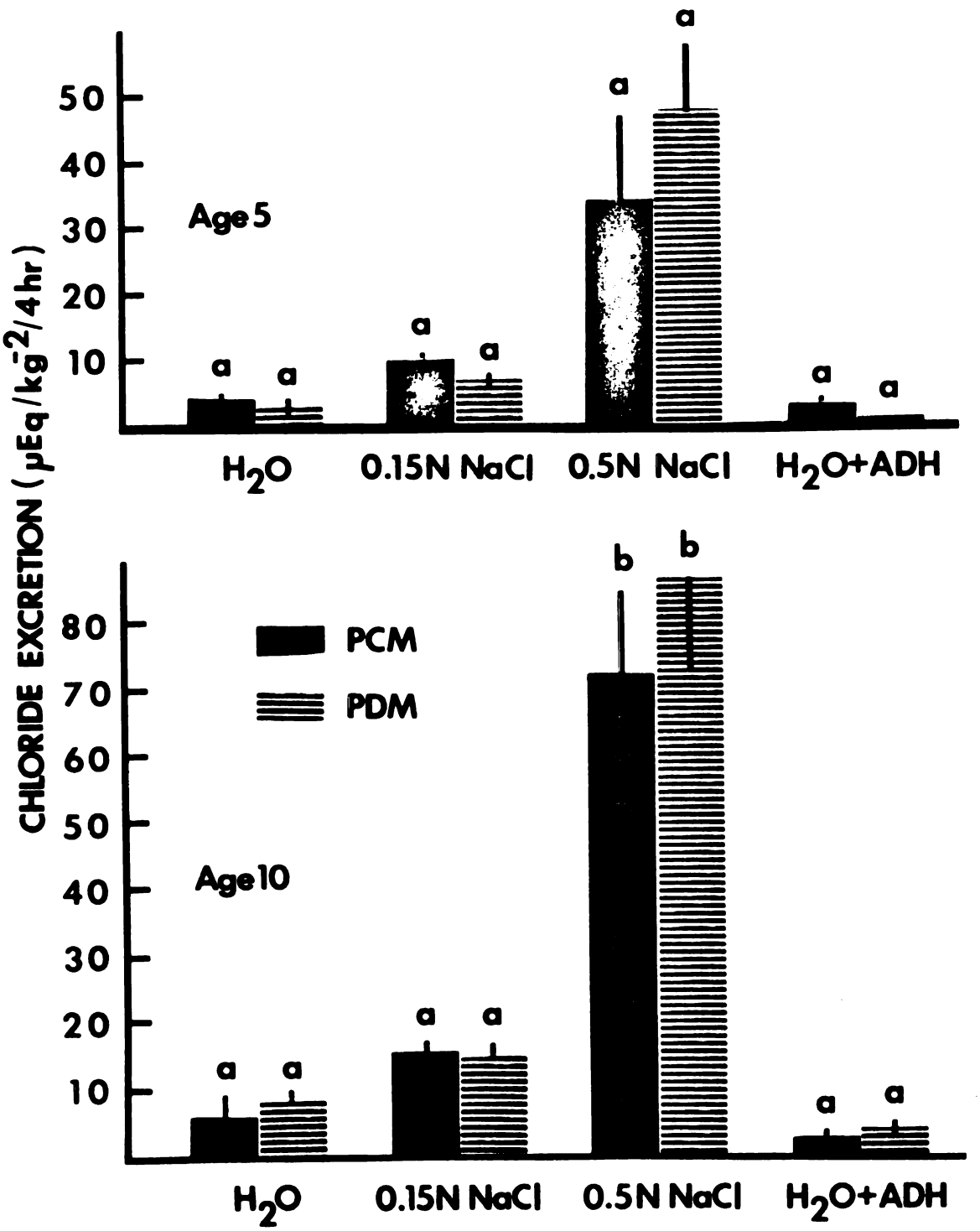

CHLORIDE EXCRETION ($\mu Eq/kg^{-2}/4hr$)
Age 5
Age 10
PCM
PDM
H_2O
0.15N NaCl
0.5N NaCl
H_2O+ADH
10
20
30
40
50
60
70
80
a
b

Figure 20. Effect of gestational diabetes on the excretion of chloride by the progeny during the 0-2 and 2-4 hour periods after salt and water loading. Rats were gavaged with water, isotonic saline (0.15N sodium chloride) or hypertonic saline (0.5N sodium chloride) at 5% of the body weight. A fourth group received the same dose of water and a subcutaneous injection of ADH (0.1 mU/g). Bladders were emptied 2 and 4 hours after treatment. Each bar represents the mean ± SEM obtained from an "n" of 4. For the 5 day old animals, urine from 2-5 rats was pooled from an "n" of 1. For 10 day old animals, urine from 1 rat was used for an "n" of 1. Means not sharing a common superscript differ significantly ($p<.05$).

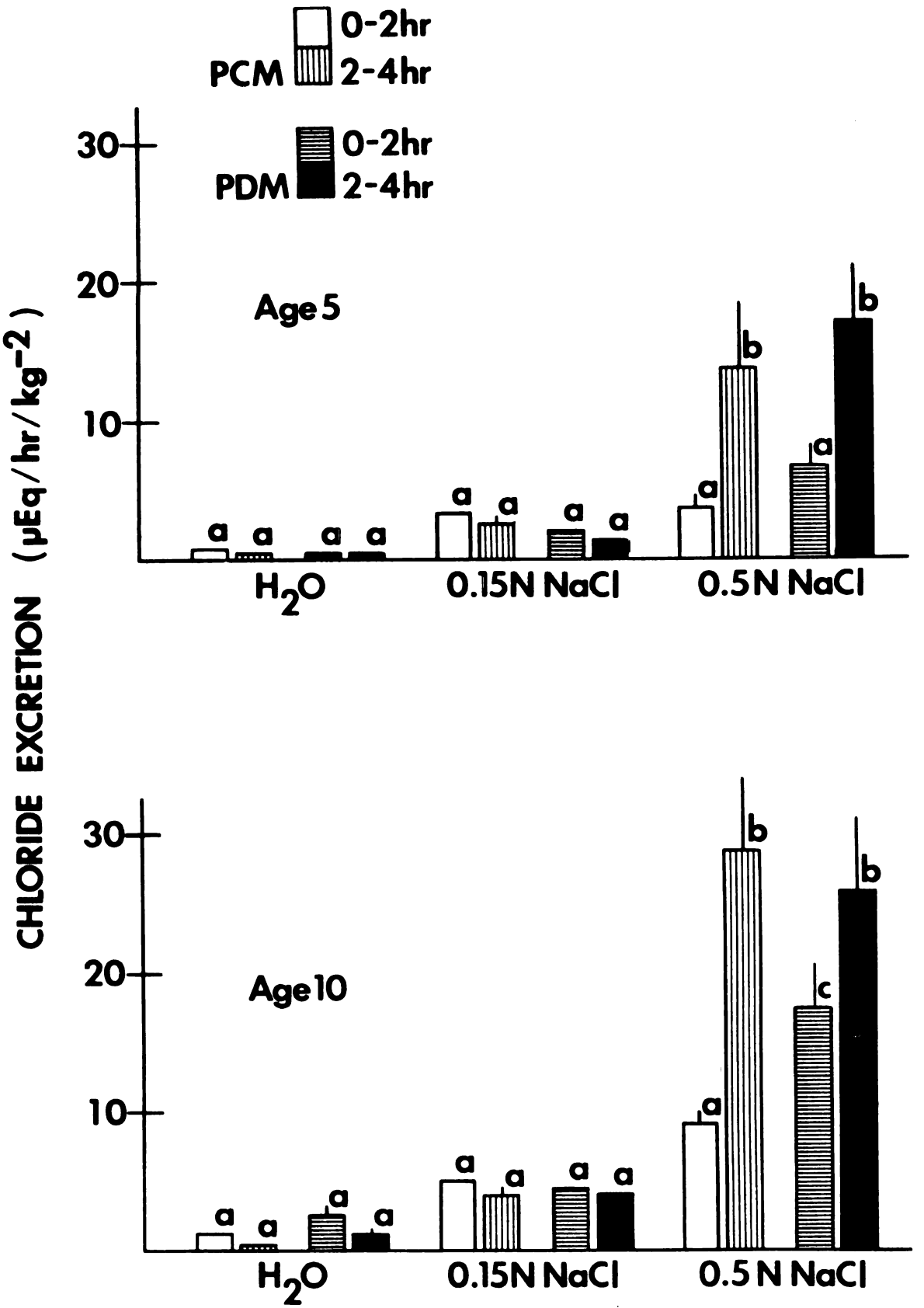

PCM
0-2hr
2-4hr
PDM
0-2hr
2-4hr
Age 5
Age 10
CHLORIDE EXCRETION (µEq/hr/kg⁻²)
30
20
10
a
b
c
H2O
0.15N NaCl
0.5 N NaCl

Figure 21. Effect of gestational diabetes on the excretion of potassium by the progeny during the first 4 hours after salt and water loading. Rats were gavaged with water, isotonic saline (0.15N sodium chloride) or hypertonic saline (0.5N sodium chloride) at 5% of the body weight. A fourth group received the same dose of water and a subcutaneous injection of ADH (0.1 mU/g). Bladders were emptied 2 and 4 hours after treatment. Each bar represents the mean ± SEM obtained from an "n" of 4. For 5 day old animals, urine from 2-5 rats was pooled for an "n" of 1. For 10 day old animals, urine from 1 rat was used for an "n" of 1. Means not sharing a common superscript differ significantly ($p<.05$).

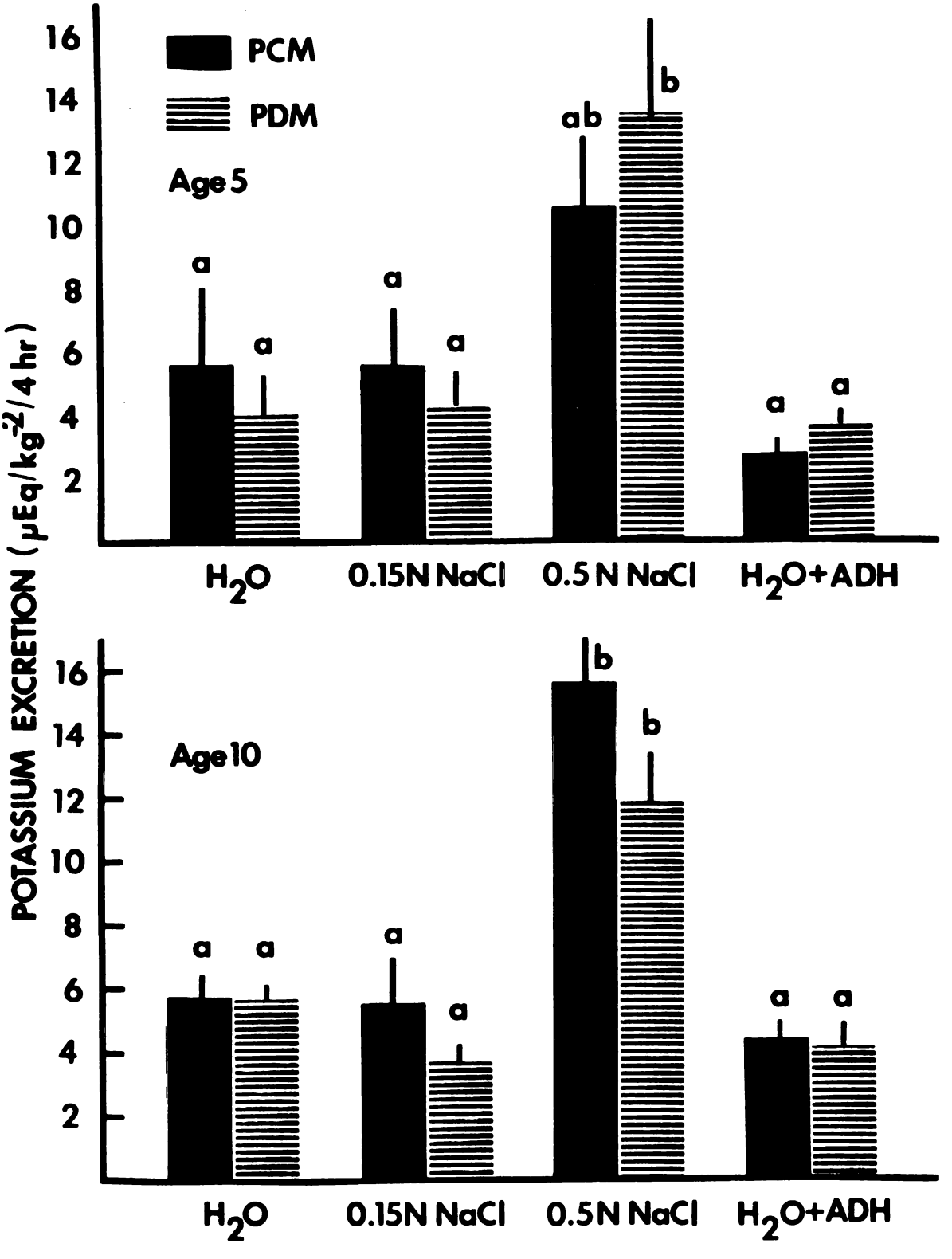

PCM
PDM
Age 5
Age 10
POTASSIUM EXCRETION (μEq/kg⁻²/4 hr)
16
14
12
10
8
6
4
2
a
a
a
a
ab
b
a
a
a
a
b
b
a
a
a
a
H2O
0.15N NaCl
0.5 N NaCl
H2O+ADH

Figure 22. Effect of gestational diabetes on the excretion of potassium by the progeny during the 0-2 and 2-4 hour periods after salt and water loading. Rats were gavaged with water, isotonic saline (0.15N sodium chloride) or hypertonic saline (0.5N sodium chloride) at 5% of the body weight. A fourth group received the same dose of water and a subcutaneous injection of ADH (0.1 mU/g). Bladders were emptied 2 and 4 hours after treatment. Each bar represents the mean ± SEM obtained from an "n" of 4. For 5 day old animals, urine from 2-5 rats was pooled for an "n" of 1. For 10 day old animals, urine from 1 rat was used for an "n" of one. Means not sharing a common superscript differ significantly ($p<.05$).

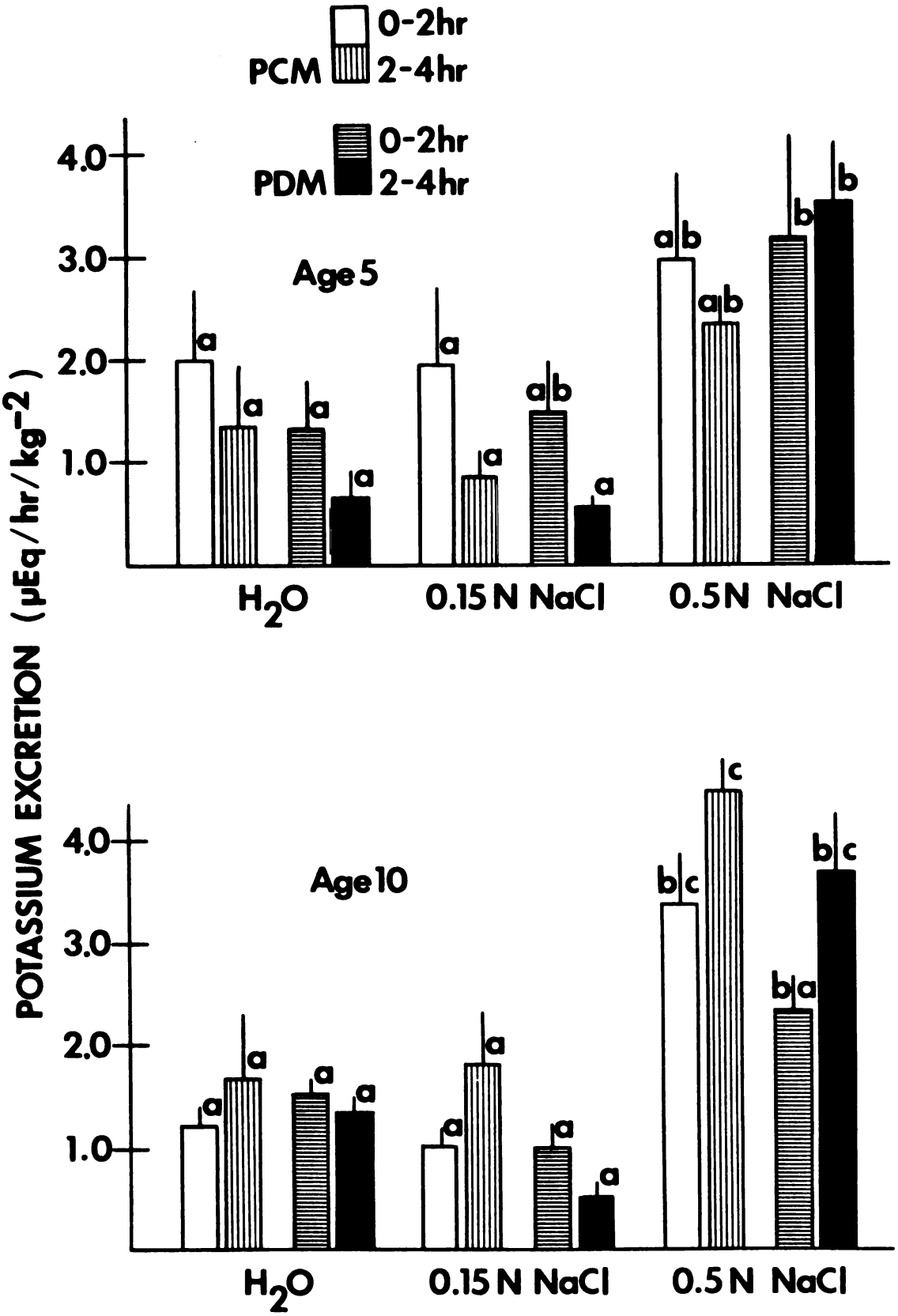

PCM
0-2hr
2-4hr
PDM
0-2hr
2-4hr
Age 5
Age 10
POTASSIUM EXCRETION (μEq/hr/kg⁻²)
4.0
3.0
2.0
1.0
H2O
0.15 N NaCl
0.5 N NaCl

Figure 23. Effect of gestational diabetes on the excretion of urea by the progeny during the first 4 hours after salt and water loading. Rats were gavaged with water, isotonic saline (0.15N sodium chloride) or hypertonic saline (0.5N sodium chloride) at 5% of the body weight. A fourth group received the same dose of water and a subcutaneous injection of ADH (0.1 mU/g). Bladders were emptied 2 and 4 hours after treatment. Each bar represents the mean ± SEM obtained from an "n" of 4. For 5 day old animals, urine from 2-5 rats was pooled for an "n" of 1. For 10 day old animals, urine from 1 rat was used for an "n" of 1.

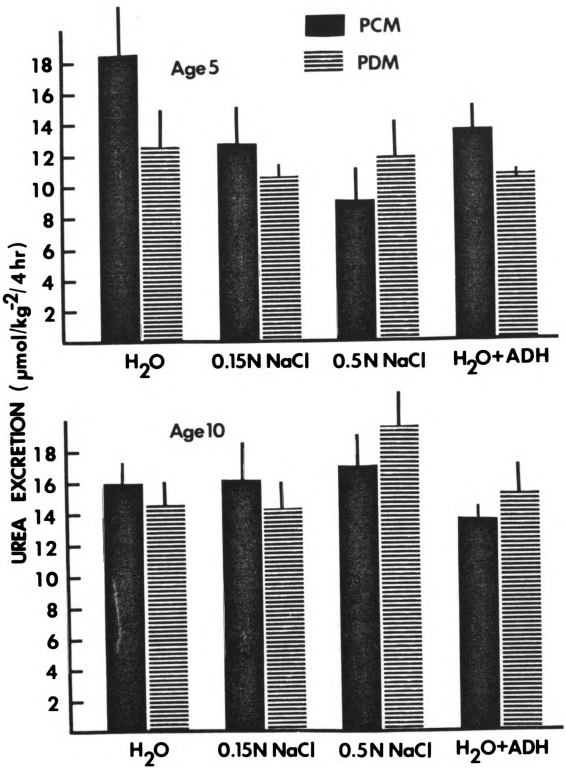

PCM
PDM
Age 5
Age 10
UREA EXCRETION (μmol/kg-2/4 hr)
2
4
6
8
10
12
14
16
18
H2O
0.15N NaCl
0.5N NaCl
H2O+ADH

Figure 24. Effect of gestational diabetes on the excretion of urea by the progeny during the 0-2 and 2-4 hour periods after salt and water loading. Rats were gavaged with water, isotonic saline (0.15N sodium chloride) or hypertonic saline (0.5N sodium chloride) at 5% of the body weight. A fourth group received the same dose of water and a subcutaneous injection of ADH (0.1 mU/g). Bladders were emptied 2 and 4 hours after treatment. Each bar represents the mean ± SEM obtained from an "n" of 4. For 5 day old animals, urine from 2-5 rats was pooled for an "n" of 1. For 10 day old animals, urine from 1 rat was used for an "n" of 1.

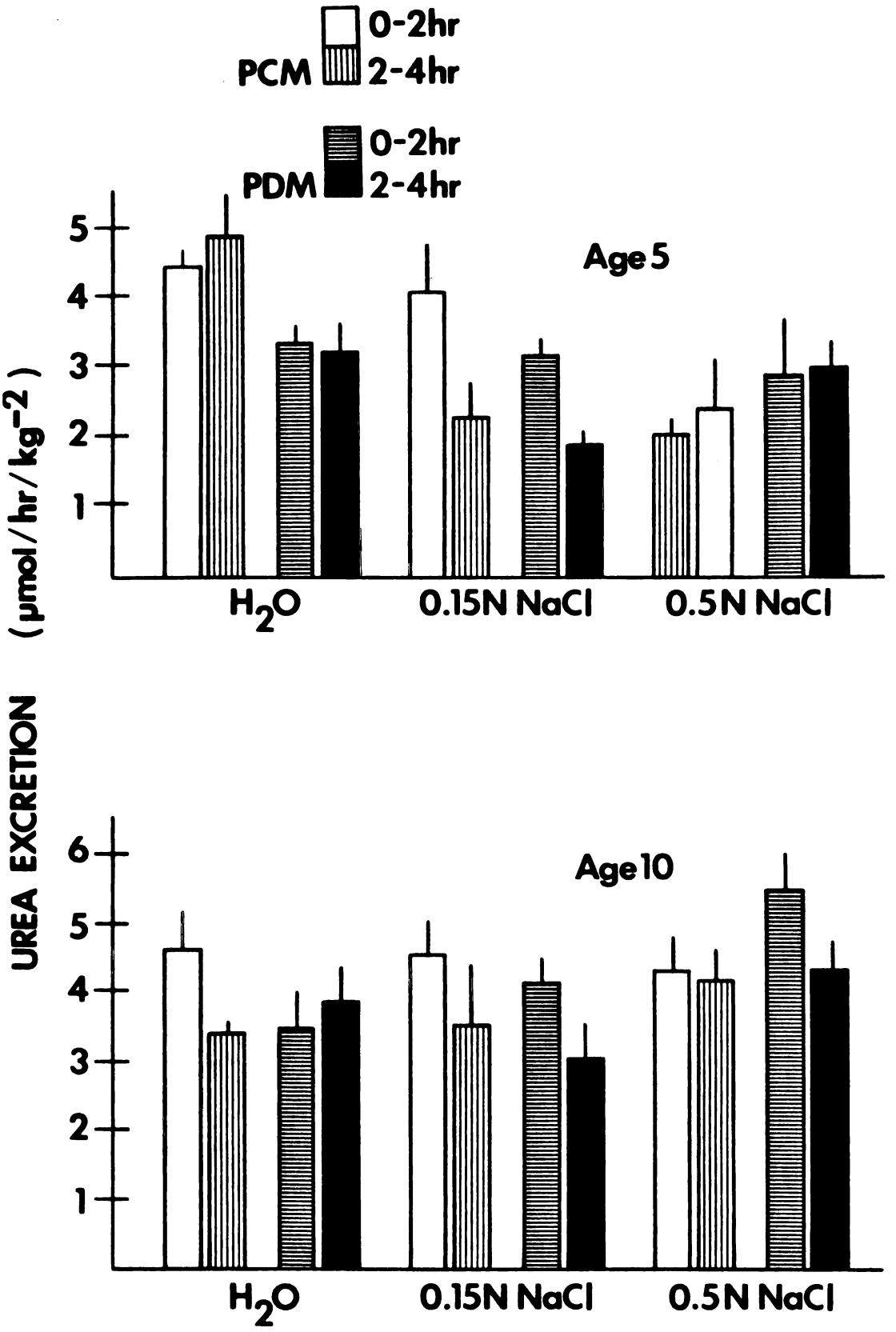

PCM
0-2hr
2-4hr
PDM
0-2hr
2-4hr
Age 5
UREA EXCRETION (μmol/hr/kg⁻²)
5
4
3
2
1
H2O
0.15N NaCl
0.5N NaCl
Age 10
6
5
4
3
2
1
H2O
0.15N NaCl
0.5N NaCl

Table 4. Response of five and ten day old PDMs and PCMs to a water load: concentration of urinary constituents

Urinary Constituent	5 Day Olds		10 Day Olds	
	0-2 Hour	2-4 Hour	0-2 Hour	2-4 Hour
Osmolality (mOsm/liter)				
PCM	145±28[a]	307±73	131±4	274±28
PDM	140±35	186±31	129±8	165±24
Urea (mM/liter)				
PCM	40±11	99±21	33±4	77±9
PDM	30±4	67±20	29±4	44±9
Sodium (mEq/liter)				
PCM	2.9±1.6	7.8±0.7	5.6±1.5	2.5±1.8
PDM	4.6±2.2	2.8±2.2	3.8±0.7	0.8±0.6
Chloride (mEq/liter)				
PCM	10.2±5	10.3±2	21.0±11	8.1±5
PDM	7.2±3	13.8±5	21.0±4	17.4±8
Potassium (mEq/liter)				
PCM	16.8±5.5	22.0±6	9.0±1	37.0±11
PDM	13.0±4.7	12.0±4	12.1±0.9	15.3±2.4

[a]Each value represents the mean ± SEM obtained from an "n" of 4. For 5 day old animals, urine from 2-5 rats was pooled for an "n" of one. For 10 day old animals, urine from one rat was used for an "n" of one. Rats were gavaged with water at 5% of the body weight. Bladders were emptied after 2 and 4 hours.

Table 5. Response of five and ten day old PCMs and PDMs to an isotonic saline load: concentration of urinary constituents

Urinary Constituent	5 Day Olds		10 Day Olds	
	0-2 Hour	2-4 Hour	0-2 Hour	2-4 Hour
Osmolality (mOsm/liter)				
PCM	433±59[a]	524±13	489±27	757±42
PDM	407±36	504±20	475±47	579±36*
Urea (mM/liter)				
PCM	88.3±5.6	93.0±5.9	99.4±9.3	134.1±25.4
PDM	86.6±12.9	92.0±7.3	97.5±13.6	94.5±10.9
Sodium (mEq/liter)				
PCM	61.1±18	122±18	101±6.9	165.5±8.6
PDM	62.5±14	92±16	67±7.5*	138.0±16
Chloride (mEq/liter)				
PCM	64.3±9.9	99.4±7.3	102.8±7.6	145±33
PDM	53.5±6.8	68.5±7.6	93.7±8.1	111±12
Potassium (mEq/liter)				
PCM	39.0±11.0	34.4±7.3	22.5±3.5	55.3±11.8
PDM	38.0±8.8	28.8±6.1	23.0±2.2	16.4±4.0*

[a]Each value represents the mean ± SEM obtained from an "n" of 4. For 5 day old animals, urine from 2-5 rats was pooled for an "n" of one. For 10 day old animals, urine from one rat was used for an "n" of one. Rats were gavaged with isotonic saline (0.15 N sodium chloride) at 5% of the body weight. Bladders were emptied after 2 and 4 hours.

*Significantly different from PCM ($p<.05$).

excreted significantly more sodium (Figure 17) and urine (Figure 13) after a hypertonic saline load than age matched PCMs.

Response of 5 and 10 Day Old PDMs and PCMs to a Water Load

Five and ten day old rats responded to a water load (5% of the body weight) with a diuresis, excreting significantly more urine than rats given an isotonic saline load (Figures 13 and 14). Both 5 and 10 day old PDMs and PCMs excreted 59% or more of the water load within the first two hours (Figure 14). Four hours after the water load, both 5 and 10 day old PCMs and PDMs excreted approximately 70% of the load (Figure 14). Two to four hours after the water load, 10 day old PDMs excreted 36% of the load while age matched PCMs excreted only 18% of the load. This was significantly different ($p<.05$).

The urinary excretion relative to body weight of total solute (Figures 15 and 16), sodium (Figures 17 and 18), chloride (Figures 19 and 20), potassium (Figures 21 and 22), and urea (Figures 23 and 24) by 5 and 10 day old PDMs after a water load was no different than that by age matched PCMs. Similarly, the concentration of solutes in the urine from 5 and 10 day old PDMs after a water load was no different than that of urine from age matched PCMs (Table 4).

Response of 5 and 10 Day Old PDMs and PCMs to an Isotonic Saline Load

In contrast to the water load, the isotonic saline load (0.15N sodium chloride, 5% of the body weight) did not produce a diuresis. A relatively small percent of the total load was excreted after 2 or 4 hours (Figure 14). Five and ten day old PDMs and PCMs excreted approximately 16-18% of the load within the first two hours and approximately 30% of the load during the entire 4 hour period (Figures 13 and 14). The excretion relative to body weight of total solute (Figures 15 and 16), sodium (Figures 17 and 18), chloride (Figures 19 and 20), potassium (Figures 21 and 22) and urea (Figures 23 and 24) by 5 and 10 day old PDMs after an isotonic saline load was no different than that by age matched PCMs.

Similarly, the concentrations of solutes in urine from 5 and 10 day old PDMs collected 2 and 4 hours after administration of isotonic saline was no different than that of urine from age matched PCMs with the exceptions of potassium and osmolar concentrations (Table 5). Urine from 10 day old PCMs, collected 2-4 hours after treatment, had a greater potassium concentration as well as a greater osmolality than urine from age matched PDMs (Table 5).

An isotonic saline load did not produce a natriuresis in 5 day old PDMs or PCMs but did produce one in 10 day old PDMs and PCMs (Figure 18).

Response of 5 and 10 Day Old PDMs and PCMs to a Hypertonic Saline Load

The osmotic diuresis produced by gavaging rats with hypertonic saline (0.5N sodium chloride; 5% of the body weight) was delayed when compared to the diuresis produced by administration of water (Figure 14). Five day old PCMs excreted 14% of the load by 2 hours and 48% of the load by 4 hours (Figure 14). Age matched PDMs excreted 22% of the load by 2 hours and 66% of the load by 4 hours (Figure 14). These were not significantly different. Ten day old PCMs excreted 18% of the hypertonic saline load after 2 hours and 66% after 4 hours (Figure 14). Age matched PDMs excreted 40% of the load after 2 hours and 95% of the load after 4 hours (Figure 14). After administration of hypertonic saline, ten day old PDMs excreted significantly more urine in the first 2 hours (Figure 14), a similar amount of urine in the second two hours (Figure 14), and significantly more urine in the entire four hour period (Figure 13) when compared to age matched PCMs. In addition, both 5 and 10 day old PDMs excreted more total solute than age matched PCMs (Figure 15). The excretion of sodium (Figures 17 and 18), chloride (Figures 19 and 20), potassium (Figures 21 and 22), and urea (Figures 23 and 24) by 5 day old PDMs was no different than that by age matched PCMs treated similarly. Ten day old PDMs treated with hypertonic saline excreted the same amount of potassium (Figure 21), chloride

(Figure 19) and urea (Figure 23) but significantly more sodium (Figure 17) within the 4 hour period than age matched PCMs treated similarly.

With the onset of the osmotic diuresis, urinary concentrations of sodium, chloride, and urinary osmolality increased while urea concentrations decreased (Table 6). Urinary potassium concentration remained unchanged in 5 day old PCMs but decreased in 5 day old PDMs with the onset of the osmotic diuresis (Table 6). The potassium concentration of urine collected from 10 day old PDMs 2 hours after the administration of hypertonic saline was significantly lower than that of urine from age matched PCMs (Table 6). In contrast, urine collected from ten day old PDMs 4 hours after treatment had the same potassium concentration as that collected from PCMs (Table 6).

In the first 2 hours after the administration of hypertonic saline ten day old PDMs excreted more urine than age matched PCMs (Figure 14) but the urine from PCMs had a significantly greater osmolality than that of PCMs (Table 6).

Response of 5 and 10 Day Old PDMs and PCMs to a Water Load in the Presence of Exogenous ADH

The excretion of urine (Figure 13), total solute (Figure 15), sodium (Figure 17), chloride (Figure 19), potassium (Figure 21), and urea (Figure 23) by 5 and 10 day old PDMs and PCMs given a subcutaneous injection of ADH

Table 6. Response of five and ten day old PCMs and PDMs to a hypertonic saline load: concentration of urinary constituents

Urinary Constituent	5 Day Olds		10 Day Olds	
	0-2 Hour	2-4 Hour	0-2 Hour	2-4 Hour
Osmolality (mOsm/liter)				
PCM	526±6[a]	550±91	674±32	565±27
PDM	481±22	485±28	525±9*	501±3
Urea (mM/liter)				
PCM	61.8±8.5	33.8±8.1	81.8±10.6	36.6±5.4
PDM	50.0±5.6	28.8±4.7	57.3±3.4	30.3±2.1
Sodium (mEq/liter)				
PCM	140±23	188±18	205±4.3	237±8.2
PDM	158±29	210±15	204±4.6	236±5.3
Chloride (mEq/liter)				
PCM	107±16	138±19	167±25	225±17
PDM	118±16	152±23	176±35	183±21
Potassium (mEq/liter)				
PCM	87±27	54±29	62.6±8.4	35.5±2.5
PDM	52±13	36±9	24.9±3.8*	27.7±2.2

[a]Each value represents the mean ± SEM obtained from an "n" of 4. For 5 day old animals, urine from 2-5 rats was pooled for an "n" of one. For 10 day old animals, urine from one rat was used for an "n" of one. Rats were gavaged with hypertonic saline (0.5N sodium chloride) at 5% of the body weight. Bladders were emptied after 2 and 4 hours.

*Significantly different from PCM ($p<.05$).

immediately after a water load (5% of the body weight) was no different than that of age matched PDMs and PCMs given only water.

Similarly, urinary concentrations of sodium, chloride potassium, urea, and urinary osmolality were no different in animals in the presence and absence of exogenous ADH (Table 7 verses Table 4).

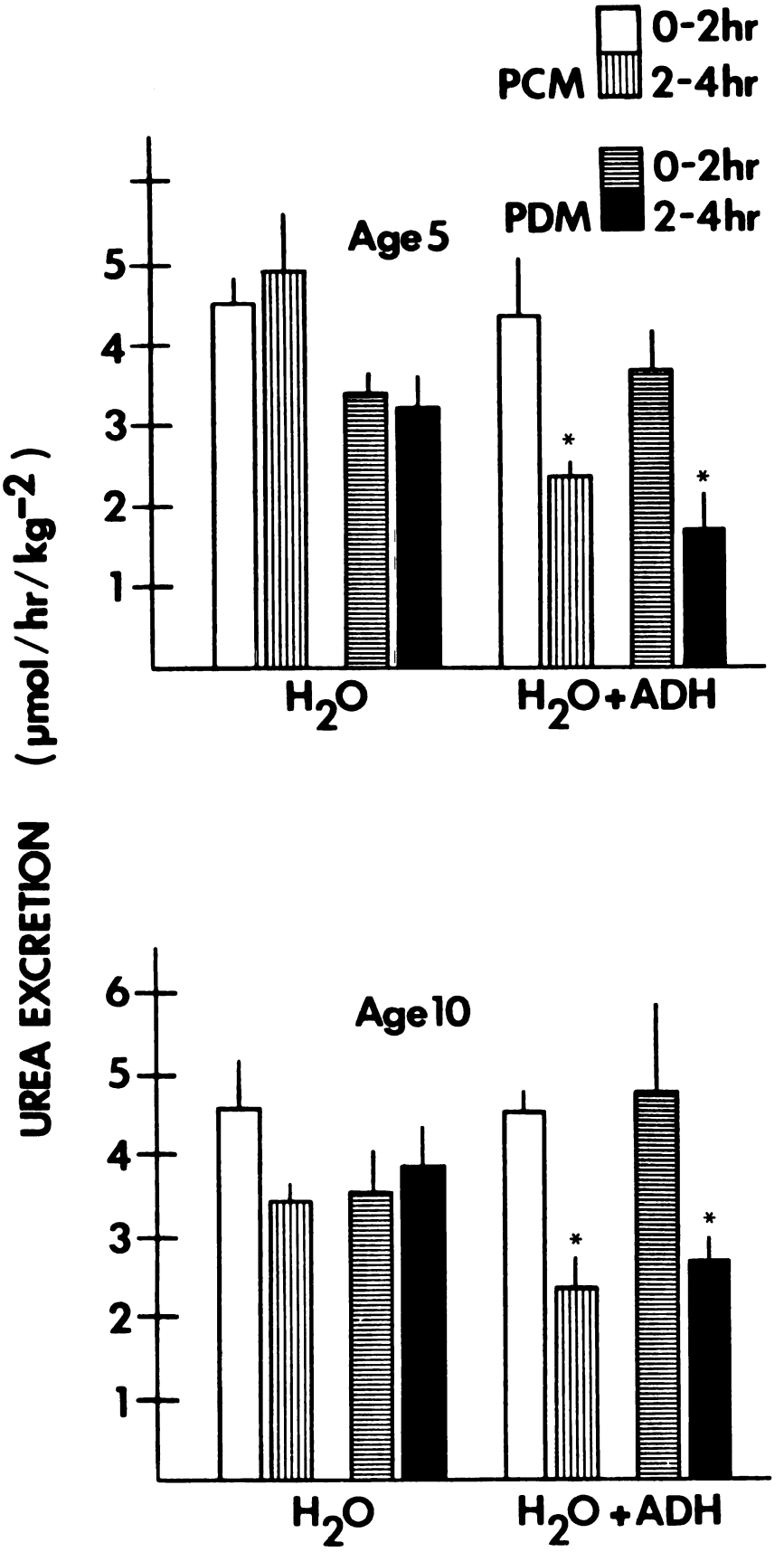
The ADH injection did, however, affect the pattern of urea excretion (Figure 25). When given exogenous ADH, both 5 and 10 day old PDMs and PCMs excreted significantly less urea during the 2-4 hour period than during the 0-2 hour period (Figure 25). In contrast, both groups of animals treated with water alone excreted the same amount of urea in the 0-2 hour and the 2-4 hour periods (Figure 25).

Table 7. Response of five and ten day old PDMs and PCMs to a water load in the presence of exogenous ADH: concentration of urinary constituents

Urinary Constituent	5 Day Olds 0-2 Hour	5 Day Olds 2-4 Hour	10 Day Olds 0-2 Hour	10 Day Olds 2-4 Hour
Osmolality (mOsm/liter)				
PCM	165±49[a]	201±32	128±5	274±20
PDM	128±18	202±17	137±14	230±61
Urea (mM/liter)				
PCM	31.6±5.2	67±11	32.0±3.6	61.6±8.7
PDM	32.6±5.2	42±3	37.9±11.7	73.0±18
Sodium (mEq/liter)				
PCM	5.0±0.8	1.5±0.8	11.8±2.3	5.0±1.2
PDM	4.0±1.1	1.4±1.4	12.6±1.9	9.5±2.5
Chloride (mEq/liter)				
PCM	8.3±3.5	11.6±5.9	7.4±2.8	6.2±4.5
PDM	3.4±1.6	3.7±2.3	15.5±6.1	1.1±1.1
Potassium (mEq/liter)				
PCM	8.5±1.4	11.1±4.4	5.0±0.9	39.5±4.6
PDM	11.2±2.7	16.1±5.1	6.3±1.1	29.1±6.0

[a]Each value represents the mean ± SEM obtained from an "n" of 4. For 5 day old animals, urine from 2-5 rats was pooled for an "n" of one. For 10 day old animals, urine from one rat was used for an "n" of one. Rats were gavaged with water at 5% of the body weight and injected subcutaneously with ADH (0.1 mU/g). Bladders were emptied after 2 and 4 hours.

Figure 25. The effect of ADH (0.1 mU/g; s.c.) on urea excretion by 5 and 10 day old PDMs and PCMs after a water load (5% body weight). Bladders were emptied 2 and 4 hours after treatment. Each bar represents the mean ± SEM obtained from an "n" of 4. For 5 day old animals, urine from 2-5 rats was pooled for an "n" of 1. For 10 day old animals, urine from 1 rat was used for an "n" of one. Asterisks denote a statistical difference from the 0-2 hour collection ($p<.05$).


PCM
0-2hr
2-4hr
PDM
0-2hr
2-4hr
Age 5
Age 10
UREA EXCRETION (µmol/hr/kg^{-2})
1
2
3
4
5
6
*
H_2O
H_2O+ADH

DISCUSSION

Newborn infants of diabetic mothers (IDMs) have a number of significant problems. As a population, they are a heterogeneous group of infants whose chemical homeostasis at birth is altered by varying degrees of hyperinsulinemia (Steinke and Driscoll, 1965; Block et al., 1974), hypoglucagonemia (Johnston and Bloom, 1975), hypocalcemia (Zetterstrom and Arnhold, 1958; Tsang et al., 1972; 1975), hypoglycemia (Farquhar, 1976; Kitzmiller et al., 1978), RDS (Farquhar, 1976; Kitzmiller et al., 1978), altered electrolyte and nitrogen excretion (Osler and Pederson, 1960; Osler, 1960; Cook et al., 1960), and metabolic acidosis (Lowery et al., 1954; Segal et al., 1957; Kitzmiller et al., 1978).

The use of rats made diabetic by alloxan or streptozotocin as an animal model for human diabetic pregnancy has led to conflicting results with respect to fetal and neonatal body weight. Aerts and Van Assche (1977) injected Wister rats on day one of pregnancy with 30, 40, or 50 mg/kg of streptozotocin to produce both mild and severe gestational diabetes. They found fetal weight of severely diabetic animals lower when compared to that of controls or mildly

diabetic animals; however, at birth there were no significant differences in body weight among rats born of control, mildly diabetic, or severely diabetic dams. Similarly, Kervran et al. (1978) produced "mild" diabetes (blood glucose ranging from 100 to 200 mg/dl) and "severe" diabetes (glucose concentration above 300 mg/dl) in pregnant rats to study the fetal pancreas. At term, fetuses from severely diabetic rats weighed significantly less than those from controls but fetuses from mildly diabetic rats weighed the same as those from controls.

Solomon (1959) injected Sprague-Dawley rats on the 8-14th day of gestation with different doses of alloxan. The mildly diabetic rats produced overweight progeny while the severely diabetic rats were unable to maintain pregnancy. In contrast, other studies have reported that untreated maternal diabetes in rats produced significantly heavier neonates while diabetic rats treated with daily injections of insulin produced normal weight progeny (Pitkin et al., 1971).

In the present study, Sprague-Dawley rats were injected with streptozotocin (50 mg/kg) on day one of gestation and treated with insulin throughout gestation in an attempt to maintain a "mild" diabetic state. Blood samples were collected on the 18th day of gestation, 24 hours after the daily insulin injection, to verify the presence of diabetes and to ascertain the severity of diabetes in the absence of

insulin control. The birth weight of the progeny did not correlate with maternal plasma glucose levels (Table 1). This finding supports clinical studies showing no direct relationship between maternal plasma glucose levels and infant birth weights (Persson, 1975). Since plasma glucose concentrations reported in the present study were determined from blood collected approximately 24 hours after insulin or saline injections, they probably do not represent the average daily plasma glucose concentration. For this reason plasma glucose levels of the dams can not be used as a means of classifying the severity of the diabetes. Since previous studies with rats have shown positive correlations between the severity of maternal diabetes, birth weight of the progeny and fetal and neonatal mortality (Solomon, 1959; Szalay and Gaal, 1975), it was of interest to examine these parameters in the present study.

The birth weights of the progeny from diabetic dams were not significantly different from those of controls (Table 1). This observation confirms some previous reports (Pitkin and Van Ordin, 1974; Aerts and Van Assche, 1977; Kervran et al., 1978) but disagrees with others observing increased weight in fetuses from mildly diabetic pregnant rats (Solomon, 1959; Pitkin et al., 1971). The reason(s) for this discrepancy remain unclear. However, a similar situation exists in the population of human IDMs. Persson (1975) reported that of 81 insulin-dependent diabetics, 56

gave birth to normal weight infants, 17 gave birth to overweight infants (greater than the 90th percentile) and 8 gave birth to underweight infants (smaller than the 10th percentile). Since mean litter birth weight for PDMs ranged from low to high (Figures 1 and 2), diabetic rats in the present study were most likely a combination of both severe and mild diabetics.

Alternatively, fetal and neonatal mortality reflect the severity of the maternal diabetes (Solomon, 1959). In the present study, the stillbirth rate from the diabetic group in Series I was 13% (Table 2). This was significantly greater than that of the control group (1.5%) and agrees with the stillbirth rate for diabetic rats reported by Solomon (1959) and by Szalay and Gaal (1975). Additionally, the neonatal death rate (percentage of pups dying within 24 hours after birth) for the diabetic group (7.5%) in Series I was greater than that of the control group (0%). Since both Solomon (1959) and Szalay and Gaal (1975) reported a higher incidence of fetal and neonatal deaths from severely diabetic rats, it is likely that the hyperglycemia in diabetic rats from Series I was not as well controlled as that in Series II. Neonatal and stillbirth rates of the diabetic group in Series II were comparable to those of control. Furthermore, there were fewer pups with low birth weights from diabetics in Series II (Figure 2) than from diabetics in Series I (Figure 1). These data suggest that the

diabetic rats in Series I represent the severely diabetic model while those in Series II represent the mildly diabetic model. This is further supported by an analysis of the frequency distribution of the body weights at birth (Figures 1 and 2). Fifty-five percent of the PDMs in Series I weighed below the grand mean (Figure 1) while 77% of PDMs weighed above the grand mean in Series II (Figure 2).

Regardless of birthweight, IDMs suffer from altered insulin metabolism. Approximately 50% of IDMs are hypoglycemic (Farquhar, 1976; Kitzmiller et al., 1978) as a result of hyperinsulinemia (Block et al., 1974). Fetuses of mildly diabetic rats showed marked hyperinsulinemia which increased in magnitude with fetal age (Kervran et al., 1978). At birth, these fetuses had significantly higher plasma insulin and glucose concentrations; birth weights, however, were not significantly different. On the other hand, neonates from severely diabetic animals had significantly depressed insulin levels, elevated blood glucose levels, and weighed significantly less than control infants (Kervran et al., 1978).

In Series I, body weights and kidney weights of rats born from diabetic dams were no different than those born of control dams at one, five, or ten days of age. However, by 28 days of age, both the body and kidney weights of PDMs were significantly less than those of age matched controls

(Figure 3 and 4). It is likely that a majority of the pups used in this set of experiments (Series I) were born from dams with severe diabetes (Figure 1, Table 2). Since neonates from severely diabetic animals have degranulated beta cells (Aerts and Van Assche, 1977) and significantly depressed plasma insulin concentrations (Kervran et al., 1978) it is possible that PDMs from Series I lacked sufficient insulin for normal growth. The observation that PDMs and PCMs weighed the same at 1, 5, and 10 days of age indicates that as long as the animals were suckling, they grew normally. When rats are approximately 14 days of age, they begin to consume the high carbohydrate rat chow (Wayne Lab Blox; 24% protein; 4% fat; 4% fiber; and 68% carbohydrate by weight) in addition to suckling. It could be that pups from severely diabetic dams could not produce sufficient insulin for use and storage of nutrients and thus, by 28 days of age, weighed significantly less than controls. Although kidney weight was lower in PDMs at 28 days of age, the protein and water content of kidney slices was no different in PDMs than PCMs at any age (Figures 5 and 6). These findings agree with data reported by Solomon (1959) and Pitkin et al. (1971), who analyzed fetal composition and found the same percentage of protein and water in fetuses born of diabetic dams as those born of control dams.

Evidence to date suggests that PDMs suffer from an altered postnatal nutritional status (Aerts and Van Assche,

1977; Kervran et al., 1978). Since alterations in postnatal nutritional status have been reported to both enhance (Bond et al., 1977) and retard (Goldstein et al., 1979) postnatal development of renal tubular transport functions in rats, it was of interest to determine the effects of gestational diabetes on the postnatal development of renal tubular transport functions in the progeny.

The intrinsic renal transport capacity of PAH from control rats 1 to 28 days of age showed a similar pattern of functional development as that reported by Kim et al. (1972) and Goldstein et al. (1979). The accumulation of PAH by renal cortical slices from control litters was low at 1 day of age and progressively increased to maximum values at 28 days of age (Figure 7). A similar pattern was observed for PDMs except at 10 days of age. At this age, renal cortical slices from PDMs accumulated significantly less PAH than those from PCMs (Figure 7).

The accumulation of PAH by renal cortical slices is age related. As the rat increases in age, so does the ability of kidney slices to accumulate PAH. Recently, Stopp and Braunlich (1977) quantified the kinetic parameters of PAH uptake by renal cortical slices using the Lineweaver-Burke analysis. These data suggest that the number of transport sites increases during postnatal development of the rat kidney. Insulin production may be abnormal in PDMs (Aerts and Van Assche, 1977; Kervran et al., 1978). Since

insulin promotes protein synthesis and since a protein carrier is responsible for PAH transport, it is possible that the depressed plasma insulin concentrations have delayed the synthesis of these protein transport sites. Thus, kidneys from 10 day old PDMs have no more transport sites than those from 5 day old PDMs. In contrast, the number of transport sites in kidney slices from PCMs increased as the animal increased from 5 to 10 days of age (Figure 7). However, as PDMs mature between 10 and 28 days of age, transport proteins are synthesized such that kidney slices from 28 day old PDMs accumulate the same amount of PAH as those from age matched controls (Figure 7).

Alternatively, the accumulation of PAH by renal cortical slices is sensitive to sodium or potassium concentration in the medium but the accumulation of NMN is not (Kim et al., 1972). Kidney slices required an ionic environment similar to the in vivo situation to accumulate PAH. This was not true for the accumulation of NMN. Since IDMs have been reported to have low tissue levels of potassium (Fee and Weil, 1963) it may be that the electrolyte composition of kidney slices from 10 day old PDMs was sufficiently altered to depress the transport of PAH.

The accumulation of the organic base, NMN, by renal cortical slices from both PDMs and PCMs 1 to 28 days of age showed the characteristic developmental pattern observed by Kim et al. (1972), and appeared to follow that of organ

growth. The ability of renal cortical slices from PDMs to accumulate NMN was the same as that of slices from PCMs (Figure 8). The accumulation of NMN by renal cortical slices appears to be less sensitive to environmental changes than that of PAH. As mentioned previously, kidney slices accumulate NMN regardless of the sodium or potassium concentration of the medium (Kim et al., 1972). Therefore, NMN S/M ratios may not represent active transport of NMN, but indiscriminate tissue binding. If this were so, the accumulation of NMN by renal cortical slices would not be altered by either changes in the electrolyte composition of kidney slices or by a lower number of functional transport proteins for NMN.

In contrast to the developmental patterns of kidney slice accumulation of PAH and NMN, the accumulation of AIB by renal cortical slices is highest at birth and progressively declines to adult values (Figure 9). These data support observations by Webber and Cairns (1968) and Segal et al. (1971). The observation that renal cortical slices from both PDMs and PCMs accumulated similar amounts of AIB probably indicates that renal tubular cells have a similar nutritive need for amino acids. AIB accumulation by renal cortical slices does not represent active reabsorption of amino acids by the tubular lumen but rather active uptake at the peritubular membrane. The fact that kidney slices from immature animals can form higher concentration gradients

or higher S/M ratios than those from adults is partly explained by a slower rate of efflux of AIB from immature kidney slices (Webber, 1968).

Unlike the accumulation of AIB, renal cortical slice accumulation of αMG does reflect active transport of sugar at the luminal membrane and thus represents the active reabsorption of sugar by the kidneys. In the present study, renal cortical slices from 1 and 5 day old controls exhibited a slight ability to concentrate αMG (Figure 10). By 10 days of age, renal cortical slices from control rats were able to accumulate αMG to maintain S/M ratios greater than 2. These data are consistent with those reported by Segal et al. (1973a) who found that renal cortical slices from newborn rats were unable to maintain a concentration gradient. Adult αMG S/M ratios of approximately 2.5 were attained by 14 days of age. The αMG S/M ratios from PDMs were similar to those of PCMs except at 10 days of age. At this age, αMG S/M ratios from PDMs were significantly less than those for PCMs (Figure 10). The accumulation of αMG, like that of PAH, reflects the number of functional transport sites (Turner and Silverman, 1977). A lower number of transport proteins resulting from alterations in insulin production by PDMs may account for low αMG S/M ratios observed in 10 day old PDMs (Figure 10).

Human IDMs have been reported to develop metabolic acidosis (Lowery et al., 1954; Segal et al., 1957;

Kitzmiller et al., 1978). The kidneys play a major role in the regulation of acid-base balance. Immature animals including humans have less ability to compensate for acid-base disturbances than adults (McCance, 1961; Goldstein, 1970). The availability of urinary buffers, such as ammonia or phosphates, determines the amount of hydrogen ion that can be excreted. The availability of urinary phosphate is limited by the serum level; however, the availability of ammonia is enhanced during metabolic acidosis by renal ammoniagenesis in the proximal tubular cells (Pitts, 1976). Thus, it was of interest to determine the ammoniagenic capacity of renal cortical slices of the progeny of diabetic dams.

Results from the present study indicate that the production of ammonia by renal cortical slices from PCMs was low (less than 200 μmol/hour/gram tissue, dry weight) at 1 day of age and remained at this level at 5 and 10 days of age. At 28 days of age, there was a marked increase in ammoniagenic capacity (400 μmol/hour/gram) (Figure 12). This pattern of development is comparable to that reported by Goldstein et al. (1979) and supports other studies in rats reporting low rates of ammonia excretion during the first 2 weeks of age and a rapid increase between 14 and 21 days of age (Goldstein, 1970; Goldstein, 1971). Similarly in the rat, renal glutaminase and glutamine synthetase levels were low, approximately one third of the adult

levels, at 9 days of age but increased progressively from 10 to 22 days of age to 70% of the adult value (Goldstein, 1971). Therefore, the dramatic increase in renal ammoniagenic capacity observed in 28 day old rats coincides with an increase in the activity of ammoniagenic enzymes (Goldstein, 1971). In the present experiments, young rats born of diabetic dams had a greatly enhanced renal ammoniagenic capacity at 1 and 5 days of age (Figure 12). Although the trend persisted at 10 days of age, the difference between PDMs and PCMs was not significantly different (Figure 12). By 28 days of age, renal cortical slices from PDMs produced the same amount of ammonia as those from PCMs.

It has been demonstrated that chronic administration of ammonium chloride to 10 day old rats enhanced the rate of ammonia excretion (Goldstein, 1970). Furthermore, it is well documented that metabolic acidosis causes an increase in both renal ammoniagenesis and gluconeogenesis in adult rats (Goodman et al., 1966; Pitts, 1976). Therefore, it is possible that prenatal and neonatal metabolic acidosis stimulated the production of ammonia by kidneys of PDMs. Fetuses of diabetic animals can develop metabolic acidosis in two ways. The first is in conjunction with the mother during diabetic ketoacidosis (Farquhar, 1976; Felig, 1977). The second occurs when fetal blood glucose concentrations increase in association with maternal hyperglycemia. Robillard et al. (1978) infused hypertonic glucose solutions

into well oxygenated fetal lambs. When fetal blood glucose concentration rose above 150 mg/dl there was a simultaneous increase in fetal plasma lactate levels and a decrease in plasma pH. When fetal plasma glucose concentrations rose above 300 mg/dl, fetal pO_2 and pCO_2 decreased and a severe metabolic acidosis ensued. Since ketonuria was not observed in any of the pregnant diabetic rats in the present study, it is unlikely that fetuses of diabetics developed ketoacidosis. However, it is probable that fetal hyperglycemia occurred and caused a lactate acidosis. A chronic acidosis throughout gestation could have increased the capacity of renal cortical slices from PDMs to produce ammonia.

Renal gluconeogenesis, like renal ammoniagenesis, is stimulated by metabolic acidosis in adult rats (Goodman et al., 1966). It is thought that renal gluconeogenesis may influence ammonia production by regulating the concentration of glutamate (Goldstein and Harley-DeWitt, 1973). In view of the effect maternal diabetes had on the renal ammoniagenic capacity of the progeny, it is interesting to consider the effects of maternal diabetes on renal gluconeogenic capacity of the progeny.

The developmental pattern for renal gluconeogenic capacity in control rats was similar to that for ammoniagenic capacity (Figure 11). That is, gluconeogenic capacity was relatively low at 1, 5, and 10 days of age but showed a marked increase at 28 days of age (Figure 11). This agrees

with the developmental pattern reported by Goldstein et al. (1979) for rats. Similarly, other studies have reported that the ability of renal cortical slices to produce glucose was significantly lower in 10 day old rats than in adults, but rose to adult levels by 12 days of age (Goldstein and Harley-DeWitt, 1973). The increase in renal gluconeogenic capacity observed to occur with increasing age does not appear to be associated with the development of gluconeogenic enzymes. Zorzoli et al. (1969) measured the activity of renal glucose-6-phosphatase (G-6-P) and PEPCK and found that adult levels were reached by 3-4 days of age. PEPCK reached a maximum of 140% of adult values at 7 days of age and thereafter declined to adult values. Therefore it appears that the increase in renal gluconeogenic capacity that occurs between 10 and 28 days of age is more closely associated with the increase in renal ammoniagenic capacity than with the development of gluconeogenic enzymes.

Renal gluconeogenic capacity in one day old PDMs was no different than that in age matched PCMs. Zorzoli et al. (1969) found that the activity of G-6-P in newborn rats was only 25% of the adult value. Similarly, the activity of PEPCK was about 50% of the adult value. Thus it could be that in one day old rats, metabolic acidosis increased renal ammoniagenesis but did not increase renal gluconeogenesis due to the limited activity of gluconeogenic enzymes at this age. However, by 5 days of age, renal cortical slices from

PDMs produced significantly more glucose from glutamate than those from age matched PCMs (Figure 11). It could be that with the increase in the activities of G-6-P and PEPCK that occurs from 2-4 days of age, these enzymes now responded to the chemical signals known to stimulate gluconeogenesis (an increase in αketoglutarate or a decrease in cytosolic pH). Since glucose production by renal cortical slices from PDMs at 10 and 28 days of age was no different than that for age matched controls (Figure 11) it appears that the stimulus that is present at 5 days of age is gone by 10 days of age.

Since it was hypothesized that fetal and neonatal acidosis induced renal ammoniagenic capacity in neonatal PDMs, it was of interest to induce an acute acidosis with intragastric administration of ammonium chloride to 10 day old PDMs and PCMs and measure subsequent renal ammoniagenic and gluconeogenic capacities.

Acute acidosis did produce a small but significant increase in gluconeogenesis (Table 3) by renal cortical slices from both PDMs and PCMs but it had no effect on renal ammoniagenesis. Goldstein and Harley-DeWitt (1973) measured the gluconeogenic capacity of renal cortical slices from 10 day old rats. In their studies, medium pH was altered to simulate <u>in vivo</u> acidosis (medium pH of 7.1). They reported that slices incubated in acidotic medium produced 22% more glucose than those incubated at pH 7.7. Slices incubated at pH 7.1 produced 61.4 ± 4.5 μmol/g dry wt x hr whereas

those incubated at pH 7.7 produced 50.3 ± 3.4. In the present experiments, all slices were incubated in medium with a pH of 7.4. Slices from acidotic animals produced 8.4% more glucose than those from controls (Table 3). Renal cortical slices from acidotic animals produced 72.8 ± 2 μmol/hr/gram dry wt while those from littermates produced 67.2 ± 1.9. Thus the values reported in this study are consistent with those reported by others (Goldstein and Harley-DeWitt, 1973) and provide evidence that alterations in medium pH, the method used by Goldstein and Harley-DeWitt (1973) did indeed simulate the *in vivo* acidotic state. Renal cortical slices from PDMs produced the same amount of glucose and ammonia as those from PCMs both in the presence and absence of acidosis (Table 3). Additionally, ammonia production by kidney slices was not enhanced by acidosis (Table 3). This observation supports data reported by many workers (Preuss *et al.*, 1974; Roobol and Alleyne, 1974; Cartier *et al.*, 1975) showing that a reduction in the pH of the medium either reduces ammonia production by kidney slices or fails to affect it, while simultaneously increasing glucose production. However, the amount of glucose and ammonia produced by both acidotic and control animals in this experiment appears higher than that produced by 10 day old rats in previous experiments (Figure 11 and 12 compared to Table 3). It is possible that all the pups were in a mild state of acidosis from lack of food. In previous

experiments, pups were taken from their mothers and immediately killed. In contrast, in this experiment, pups were kept from their mothers for 3 to 4 hours before they were killed. While this mild state of acidosis may mask the effects of the ammonium chloride on renal ammoniagenesis and gluconeogenesis, it probably would not mask differences between PDMs and PCMs. Acidosis remains a possible cause for the increased ammoniagenic capacity observed in one and five day old PDMs. However, renal ammonia production can be influenced by other factors. Recent studies have shown that in vitro renal ammoniagenesis appears to be regulated by substrate oxidation by kidney slices. When kidney slices are incubated in a medium containing lactate alone or combined with other fuels, renal ammoniagenesis is markedly inhibited. In addition, the presence of lactate causes an increased accumulation of glutamate. In contrast, the inhibition of ammoniagenesis or the accumulation of glutamate does not occur when kidney slices come from acidotic rats (Preuss et al., 1978a). Thus it appears that lactate alone or combined with other substrates decreases ammoniagenesis primarily at the GDH step and that slices from acidotic rats are resistant to substrate mediated inhibition.

Alternatively, potassium depletion is associated with high rates of ammonia excretion (Tannen, 1977). Since low plasma potassium concentrations have been reported for some IDMs (Farquhar, 1976), potassium depletion may also be a

factor in the enhanced ammoniagenic capacity observed in PDMs.

IDMs have been reported to excrete abnormally large amounts of urine, potassium, chloride, and nitrogen (Osler, 1960; Cook et al., 1960; Osler and Pederson, 1960). Since abnormal electrolyte excretion may reflect abnormal kidney function among other things, it was of interest to determine the ability of PDMs to compensate for salt and water excesses.

The results indicate that 5 and 10 day old PDMs and PCMs responded in a similar way to a water load. PDMs and PCMs gavaged with water (5% of body weight) responded by increasing volume (Figure 13 and 14) and decreasing concentration (Table 4) of urine. Approximately 50% of the water load was excreted by 2 hours and 70% by 4 hours after treatment. The results for the control group agree with those reported by McCance and Wilkinson (1947) and Falk (1955) and Allen and Zeman (1973). Similarly, the concentrations of urea and the osmolality of urine during a water diuresis reported in the present study agree with those reported by McCance and Wilkinson (1947) for 4 to 12 day old rats.

Since the total excretion of urine by PDMs was no different than that by PCMs (Figure 13) in response to a water load, it is difficult to explain the observation that 10 day old PDMs excreted more urine in the 2-4 hour period than age matched PCMs. Since Falk (1955) and McCance and

Wilkinson (1947) reported that the older the rat, the greater the percent of the water load it was able to excrete, it is possible that the continuation of a water diuresis into the 2-4 hour period by PDMs indicates that PDMs have a more mature response to a water load than PCMs. Alternatively, the secretion of or response to ADH can affect water excretion.

ADH increases the permeability of the distal tubular and collecting duct membranes to water and thus increases water reabsorption. The degree to which water is reabsorbed is, in turn, dependent on the hypertonicity of the medullary interstitum. Since IDMs have been reported to excrete more urine than INMs (Osler, 1960) a decreased production of ADH or a decreased sensitivity to ADH by the kidneys would result in a greater urine flow. In this light, it is interesting to examine the effects of exogenous ADH on the ability of PDMs and PCMs to excrete a water load.

A subcutaneous injection of ADH (0.1 mU/g body weight) given in conjunction with water (5% of body weight) administered by gavage had no effect on the subsequent water diuresis in 5 and 10 day old PDMs and PCMs (Figures 13, 15, 17, 19, 21, 23). This is in contrast to data reported by Falk (1955) who found that rats treated with ADH as young as 3 days of age, excreted half as much urine as nontreated animals after a water load. Similarly, Falk found that exogenous ADH caused an increase in urinary chloride

concentration and urinary osmolality. Alternatively, the results reported in the present study agree with those reported by Heller (1947) who did not show an inhibitory effect of ADH on a water diuresis until rats were 4 weeks old. Falk injected the ADH one-half hour after the administration of water and collected urine samples every half hour while Heller measured the cumulative excretion over a period of hours. Allen and Zeman (1973) found that ADH depressed urine flow in 6 day old rats between 30 and 60 minutes after a water load but ADH did not reduce urine flow from 60 to 180 minutes after the water load.

The results reported in the present study represent the cumulative urine collection over 2 hours. Therefore it could be that ADH depressed urine flow in the first hour, a water diuresis ensued in the second hour, and when bladders were emptied at 2 hours, the initial urine was diluted by the subsequent diuresis. Thus the final concentration was no different than if the animal had not received ADH. Interestingly, all animals injected with ADH showed an altered pattern of urea excretion (Figure 25). This is especially intriguing since previous studies with ADH show that it acts primarily during the first hour after the injection (Allen and Zeman, 1973). The excretion of urea was no different in the first 2 hours, but was significantly depressed in the second two hours (Figure 25). Although the concentration of urinary urea varied significantly among the

treatments, the excretion of urea was no different among the treatments, indicating that the excretion of urea was not affected by the diluting mechanism of the kidney. The reason for a depressed urea excretion 2-4 hours after the administration of ADH is not known.

A water load distributes into all the cellular compartments, decreasing the osmolality of the tissues and increasing the volume. Both baroreceptors and osmoreceptors are stimulated and cause a decrease in ADH secretion. This causes a decrease in the permeability of the membranes of the distal tubules and collecting ducts to water and results in a water diuresis. An isotonic saline load, on the other hand, distributes only to the extracellular fluid compartment. There is no change in osmolality of the fluid but there is an increase in volume. In response to an isotonic saline load, it has been demonstrated that renal tubular sodium reabsorption is decreased and that this decrease is not affected by aldosterone, ADH, or a decreased GFR (Pitts, 1976). Micropuncture studies have demonstrated that the natriuresis observed in adult rats infused with isotonic saline is a result of reduction in both proximal and distal tubular sodium reabsorption (Davis et al., 1969).

The results of the present study indicate that an isotonic saline load (0.15N NaCl; 5% body weight) did not produce a diuresis in 5 or 10 day old PDMs or PCMs. Four hours after the administration of an isotonic saline load,

only 30% of the load had been excreted (Figure 14). Urine excretion 0-2 and 2-4 hours after administration of isotonic saline was significantly less than that after water or hypertonic saline loads (Figure 14). These results are similar to those reported by Hoy (1966) who administered water, hypertonic saline, and isotonic saline intravenously and intragastrally to newborn rats and measured cumulative urine flow and urinary chloride concentrations over 2 hours. The administration of isotonic saline had no effect on urinary chloride concentration after 2 hours. The urinary chloride concentrations reported by Hoy (1966) are similar to those reported in the present experiments.

Although the administration of isotonic saline did not produce a diuresis, it appeared to affect the concentration of urinary solutes 4 hours after treatment (Table 5). Compared to pretreatment values (Appendix A) urinary osmolality, sodium, and chloride concentrations appeared to increase, urea concentration remained unchanged, and potassium concentration appeared to decrease. The fact that urea concentration remained the same as pretreatment values (Appendix A) indicates that urine flow after an isotonic saline load was no different than it was before the load. In contrast, after a hypertonic saline or water load, the urea concentration decreased as the water or osmotic diuresis ensued.

Five day old PDMs responded to an isotonic saline load in the same manner as 5 day old PCMs (Figures 13 to 24). Ten day old PDMs excreted the same amount of urine (Figures 13 and 14) and urinary solutes (Figures 15 to 24) in response to isotonic saline as age matched PCMs; however, the osmolality as well as the potassium concentration of urine from PDMs collected during the 2-4 hour period after the administration of isotonic saline was significantly lower than that of urine from PCMs (Table 5). Similarly, the sodium concentration of urine collected 0-2 hours after treatment from PCMs was significantly greater than that from PDMs. Since the excretion of these solutes is no different between the two groups, urine flow must be slightly increased in PDMs during these periods (Figure 14).

According to Pitts (1976), a water diuresis unrelated to change in osmolality of the body fluids can be induced in adults by infusion of large volumes of isotonic saline. Supposedly, volume and pressure receptors are stimulated by the excess of extracellular volume. The excretion of water is compensatory in that it reduces extracellular fluid volume. Unless accompanied by the excretion of sodium, loss of volume is limited by increasing osmolality of the body fluids. In adults, enhanced sodium excretion (natriuresis) is readily induced by infusions of isotonic saline (Pitts, 1976). Kleinman and Hsieh (1974) found that saline loading produced a decrease in proximal tubular sodium

reabsorption in both adult and newborn dogs and a decrease in distal tubular sodium reabsorption in adults. In contrast, there was an increase in distal tubular sodium reabsorption in newborn dogs. This may partly explain the observation that newborn animals do not excrete a saline load to the same extent as adults.

Although the urinary sodium concentration rose after isotonic saline administration a natriuresis did not occur in 5 day old rats since the excretion of sodium was no different than that of animals receiving water. By 10 days of age, however, an isotonic saline load produced a natriuresis in both PDMs and PCMs (Figure 14).

A hypertonic saline load administered by gavage to 5 and 10 day old rats produced an osmotic diuresis. Compared with a water diuresis, this was delayed since maximum urine excretion occured 2-4 hours after treatment rather than 0-2 hours after treatment (Figure 14). Additionally, when compared to the water or isotonic saline load, the hypertonic saline load produced a significant increase in the total excretion of sodium (Figure 17) and chloride (Figure 19) in all rats and the total excretion of potassium (Figure 21) and total solute (Figure 15) in 10 day old rats. Urea excretion was no different after a hypertonic saline load than after a isotonic saline or water load (Figure 23).

The response by 5 and 10 day old rats to a hypertonic saline solution reported in the present study is similar to

that reported by Falk (1955) for 4-8 day old rats. Falk gavaged 4-8 and 11-13 day old rats with 5% of the body weight of hypertonic saline. He found no significant difference in the ability to excrete a hypertonic saline load between the 4-8 and 11-13 day olds. Furthermore, he found that urine flow and urinary chloride concentration increased rapidly in all groups, however, the increase in urine flow by rats less than 3 weeks of age was delayed when compared to adults. The urinary chloride concentrations (Table 6) in response to hypertonic saline agree with those reported by Falk (1955) and Hoy (1966). These researchers did not report concentrations of other urinary electrolytes.

Ten day old PDMs and PCMs excreted significantly more urine in response to hypertonic saline than to isotonic saline (Figures 13 and 14). Ten day old PDMs excreted significantly more urine than age matched PCMs. At 5 days of age, PDMs treated with hypertonic saline excreted more urine than those treated with isotonic saline but PCMs did not. Furthermore, PDMs excreted significantly more urine during the first 2 hours after treatment than PCMs. Since Falk (1955) found that the older the rat, the more rapidly it responded to a hypertonic saline load, these data could indicate that 10 day old PDMs excreted a hypertonic saline load in a more mature fashion than PCMs. Similarly, both 5 and 10 day old PDMs excreted more total solutes than age matched PCMs in response to hypertonic saline (Figure 15).

In 10 day old PDMs, this was due to the significant increase in urine flow during the first 2 hours since the urine from PDMs had lower osmolality than that of PCMs (Table 6). In view of the fact that solute excretion by PDMs after a hypertonic saline load was greater than that by PCMs, it was interesting to examine the major urinary constituents that are osmotically active. These are sodium, chloride, potassium and urea.

As with solute excretion, total sodium excretion by both 5 and 10 day old rats was greater in animals treated with hypertonic saline when compared to those treated with isotonic saline or water (Figure 17). At 10 days of age, the response by both PDMs and PCMs was greater than that of the 5 day old counterparts. Ten day old PDMs excreted more sodium than age matched PCMs. Since there was no significant difference in the urinary sodium concentration, the greater excretion of sodium by PDMs is probably due to increased urine flow (Figure 13). This is apparent when the pattern of urine flow is examined. PDMs excreted 2 times more urine in the first 2 hours after treatment than PCMs (Figure 14). Similarly, PDMs excreted 2 times more sodium in the first 2 hours than PCMs (Figure 18). Urine excretion as well as sodium excretion by PDMs and PCMs during the 2-4 hour period after treatment were not significantly different. Therefore, one possible reason that PDMs excreted more sodium over the 4 hour period than PCMs

(Figure 17) is a more rapid onset of an osmotic diuresis.

The chloride excretion by 10 day old rats in response to a hypertonic saline load was similar to sodium excretion (Figure 20, Table 6). This was not true for 5 day old animals. At 5 days of age there was no difference in the total chloride excreted by animals gavaged with water, isotonic saline, or hypertonic saline. Chloride excretion after the first two hours was the same regardless of treatment (Figure 20). However, by 2-4 hours after treatment, 5 day old rats excreted significantly more chloride in response to hypertonic saline than in response to isotonic saline or water (Figure 20). The increase in chloride excretion is caused by the significant increase in urine flow which occurred during this period (Figure 14). These data agree with those reported by Falk (1955) who found that urinary chloride concentration in 4-8 day old rats treated with hypertonic saline was less than 100 mEq/liter by 1 hour, approximately 200 mEq/liter by 2 hours, and maintained at 200 mEq/liter until 4 hours after treatment. Chloride excretion by 10 day old rats was similar to that described for sodium. PDMs excreted significantly more chloride in the first two hours after treatment with hypertonic saline than PCMs (Figure 20). Again, since urinary chloride concentration was not significantly different between PDMs and PCMs (Table 6) this increased excretion can be explained by the increased urine excretion observed

during this period (Figure 14).

Total potassium excretion by 10 day old rats was significantly greater in animals treated with hypertonic saline than those treated with isotonic saline or water (Figure 21). It is well established that an osmotic diuresis in adult animals increases the excretion of potassium, but a water diuresis does not (Pitts, 1976). The results from the present experiments indicate that the response of an increased excretion of potassium in conjunction with an osmotic diuresis develops sometime between 5 and 10 days of age in rats (Figure 21). This response, however, appears to have developed earlier in PDMs. Five day old PDMs treated with hypertonic saline excreted more potassium than those treated with water whereas age matched PCMs treated similarly excreted the same amount of potassium as those treated with water (Figure 21).

Filtered potassium is largely reabsorbed in the proximal tubule and the amount of potassium presented to the tubule varies only slightly despite variation in urinary potassium. Changes in potassium secretion by the distal tubules determine changes in potassium excretion. Potassium secretion by the distal tubule is dependent on the delivery of sodium to the distal nephron. The more sodium ions that diffuse across the luminal membrane into the cell, the higher will be the distal transepithelial potential difference. It is the reabsorption of sodium that is responsible

for the electronegativity of the tubular fluid and this is responsible for maintaining the electrochemical driving force for potassium ions to enter the tubular lumen (Pitts, 1976). Thus, during an osmotic diuresis, delivery of sodium to the distal tubule is increased, sodium reabsorption is increased and the transepithelial potential is increased causing an increase in potassium secretion and an increase in potassium excretion.

While there were no differences in potassium excretion by PCMs and PDMs gavaged with hypertonic saline, the concentration of potassium in urine collected from PDMs 2 hours after treatment was significantly less than that from PCMs (Table 6). This can be explained by the fact that urine flow of PDMs during this time was significantly greater than that of PCMs and indicates that the onset of the osmotic diuresis began earlier in PDMs than PCMs (Figure 14).

Urea excretion by animals treated with hypertonic saline was no different than that by animals treated with isotonic saline or water (Figures 23 and 24). Both during the water diuresis and the osmotic diuresis, urea concentration of the urine decreased (Tables 4 and 6). After an isotonic saline load, the urea concentration of the urine remained the same as pretreatment values (Appendix A). This supports previous evidence concerning the renal handling of urea in adult rats (Lassiter et al., 1961; 1964). Urea

is the main end product of nitrogen metabolism and its excretion can be influenced by the amount of protein ingested (Addis and Drury, 1923). It undergoes 2 processes in the kidney, glomerular filtration and tubular reabsorption. Micropuncture studies have demonstrated that tubular reabsorption of urea is passive and dependent on the concentration gradients established by water reabsorption (Lassiter et al., 1961; 1964). Therefore, during either a water or osmotic diuresis in mature rats, the urea excretion remains constant while its urinary concentration decreases.

The results of the present study indicate that 10 day old PDMs excreted a greater percent of the hypertonic saline load than age matched PCMs (Figures 13 and 14). In response to such a load, PDMs excreted a greater quantity of total solute (Figure 15) which may be explained by an increase in sodium excretion (Figure 17) relative to body weight, when compared to age matched PCMs. Sodium excretion is the net result of the sodium filtered minus the sodium reabsorbed. Therefore, PDMs could have a greater GFR or a lower tubular reabsorption of sodium. Adult rats can excrete greater quantities of hypertonic saline more rapidly than can infants (Falk, 1955). Therefore it appears that PDMs have demonstrated a more mature renal response to a hypertonic saline load than PCMs. It is unlikely that this enhanced renal function is due to postnatal nutrition since PDMs and PCMs receiving the same treatments were suckled by the same dam. Since a

majority of rats used in this series of experiments (Series II) had birth weights above the grand mean (Figure 2), it is possible that the enhanced renal function observed is a result of prenatal overnutrition. Bond et al. (1977) showed that 5 and 10 day old rats born of dams with one ligated uterine horn had enhanced renal secretory capacities for organic ions. Alternatively, Hall and Zeman (1968) and Allen and Zeman (1973) demonstrated that prenatal protein malnutrition severely depressed GFR as well as the ability to excrete both water and osmotic loads in the progeny. Therefore, it is possible that a mild gestational diabetes in rats enhanced renal development in the progeny.

The results of these experiments indicate that gestational diabetes modified the development of certain kidney functions in rats. Compared to normal development, the development of intrinsic renal tubular transport functions appeared to be relatively unaffected, the development of renal gluconeogenic and ammoniagenic capacities were altered, but not permanently, and the development of the ability to excrete a water or isotonic saline load was not affected. The ability to excrete a hypertonic saline load appeared to be enhanced by gestational diabetes.

In light of this information, many questions can be raised. What changes in body chemistry caused the increased renal ammoniagenic capacity observed in neonatal PDMs? What hormonal or renal tubular changes are involved with the

enhanced ability to excrete hypertonic saline?

To avoid complications, IDMs are frequently delivered prematurely (Farquhar, 1976). According to a recent survey (Kitzmiller et al., 1978), IDMs were delivered from the 28th to the 39th week of gestation. The kidneys of fetuses delivered before 36 weeks of gestation would be in the process of nephrogenesis and, in that sense, similar to the kidneys of neonatal rats. Therefore, studies concerning the development of renal function in PDMs may help to more fully understand the ability of human IDMs to cope with the alterations in the chemical homeostasis that occur shortly after birth.

SUMMARY

The effect of gestational diabetes on the development of kidney function in the progeny was determined using *in vitro* slice techniques and *in vivo* excretion studies.

Female rats were injected on day one of gestation with streptozotocin (50 mg/kg) and were given daily insulin injections throughout pregnancy. At birth, pups from diabetic mothers (PDMs) and pups from control mothers (PCMs) were cross fostered to lactating controls.

Two series of experiments were completed. In Series I, body weight, kidney weight, kidney cortex composition and kidney function were assessed at 1, 5, 10, and 28 days of age. Renal function was quantified using the *in vitro* kidney slice technique. In Series II, ability to excrete water, isotonic saline and hypertonic saline loads was assessed at 5 and 10 days of age.

In Series I, significantly more PDMs were born dead or died within 24 hours after birth than PCMs. Body weight and kidney weight of PDMs were no different than those of PCMs at 1, 5, or 10 days of age, but were significantly lower at 28 days of age. Newborn rats of severely diabetic dams have low blood insulin levels (Kervran *et al.*, 1978)

and beta cell degranulation (Aerts and Van Assche, 1977). Thus growth retardation observed in PDMs may be related to depressed insulin secretion.

The percentage of water or protein in the kidney cortex from PDMs was no different than that of PCMs at 1, 5, 10, or 28 days of age.

Renal tubular transport functions were quantified utilizing the slice technique described by Cross and Taggart (1950). Accumulation of a prototype organic acid (PAH), organic base (NMN), amino acid (AIB) or sugar (αMG) by renal cortical slices was expressed as a slice/medium (S/M) ratio.

Accumulation of PAH by renal cortical slices from PCMs was lowest at day one of age and increased from 1 to 28 days of age. For PDMs, PAH S/M ratios were similar to those observed for PCMs except at 10 days of age when they were significantly lower. Similarly, accumulation of αMG by renal cortical slices from PCMs was lowest at birth and increased with age. For PDMs, αMG S/M ratios were similar to those reported for PCMs, except at 10 days of age, when they were significantly depressed. It was suggested that lower insulin levels in PDMs compromised protein synthesis and that this resulted in a lower number of functional transport sites for sugars or organic acids in ten day old PDMs when compared to age matched PCMs.

Accumulation of NMN by renal cortical slices from PDMs was similar to that of PCMs. NMN S/M ratios were lowest at

birth and increased with age. Accumulation of NMN by renal cortical slices is not sensitive to ionic changes in the medium (Kim et al., 1972) and may represent indiscriminate tissue binding.

Accumulation of AIB by renal cortical slices from PDMs was no different than that from PCMs. AIB S/M ratios were highest at birth and decreased with age.

Renal ammoniagenic or gluconeogenic capacity of PCMs was low at 1, 5, and 10 days of age but significantly increased by 28 days of age. Renal ammoniagenic capacity of PDMs was significantly greater than that of PCMs at 1 and 5 days of age but declined to normal values by 10 and 28 days of age. Renal gluconeogenic capacity of PDMs was the same as that for PCMs at 1, 10, and 28 days of age. At 5 days of age, renal cortical slices from PDMs produced significantly more glucose than those from PCMs. Since fetal hyperglycemia leads to a lactate acidosis (Robillard et al., 1978) it was suggested that a mild chronic acidosis in the fetus or neonate enhanced renal ammoniagenesis in newborn PDMs.

To produce acidosis, ten day old PDMs and PCMs were gavaged with ammonium chloride (5 mM/kg; 2.5% of body weight). Approximately 3 hours later, renal cortical slices from acidotic rats produced slightly more glucose but no more ammonia than those from non-acidotic rats. Renal cortical slices from PDMs produced the same amount of glucose

and ammonia as those from PCMs.

In Series II the stillbirth and neonatal death rates were similar for PDMs and PCMs. To assess kidney function, the ability to excrete salt and water loads was determined in 5 and 10 day old PDMs and PCMs. Rats were given one of 4 treatments (5% body weight of water; 5% body weight of isotonic saline; 5% body weight of hypertonic saline; and 5% body weight of water in the presence of exogenous ADH) and bladders were emptied by perineal stimulation after 2 and 4 hours. Urine volume, electrolytes, nitrogen, and osmolality were determined. The urinary response to a water or isotonic saline load was similar in 5 or 10 day old PDMs and PCMs. In contrast, 10 day old PDMs excreted more urine, total solute, and sodium in response to a hypertonic saline load than PCMs. Since the ability to excrete hypertonic saline increases as the animal matures, these data may indicate that PDMs have a more mature renal response to hypertonic saline than PCMs.

APPENDIX

APPENDIX A

Table 8. Urinary solute concentration before salt and water loading

	PCM	PDM
Osmolality (mOsm/liter)	406 ± 16 (4)[a,b]	376 ± 42 (4)
Urea (mM/liter)	89.4 ± 5.1(4)	80.0 ± 11 (4)
Sodium (mEq/liter)	19.3 ± 5.0(8)	26.0 ± 6.0(8)
Chloride (mEq/liter)	59 ± 12 (8)	57 ± 15 (8)
Potassium (mEq/liter)	62.3 ± 4.5(8)	64.5 ± 6.9(8)

[a]Each value represents the mean ± SEM (n).

[b]Urine samples were collected from 5-6 days old rats.

LIST OF REFERENCES

LIST OF REFERENCES

Addis, T. and Drury, D.R. The rate of urea excretion. VII. The effect of various other factors than blood urea concentration on the rate of urea excretion. J. Biol. Chem. 55:629-638, 1923.

Aerts, L. and Van Assche, F.A. Rat foetal endocrine pancreas in experimental diabetes. J. Endocrinol. 73: 339-346, 1977.

Allen, L.H. and Zeman, F.J. Influence of increased postnatal food intake on body composition of protein deficient rats. J. Nutr. 101:1311-1318, 1971.

Allen, L.H. and Zeman, F.J. Influence of increased postnatal nutrient intake on kidney cellular development in progeny of protein deficient rats. J. Nutr. 103: 929-936, 1973(a).

Allen, L.H. and Zeman, F.J. Kidney function in the progeny of protein deficient rats. J. Nutr. 103:1467-1478, 1973(b).

Alleyne, G.A.O. and Scullard, G.H. Renal metabolic response to acid base changes. I. Enzymatic control of ammoniagenesis in the rat. J. Clin. Invest. 48:364-369, 1969.

Alleyne, G.A.O. Renal metabolic response to acid-base changes. II. The early effects of metabolic acidosis on renal metabolism in the rat. J. Clin. Invest. 49: 943-951, 1970.

Aschinberg, L.D., Goldsmith, D.I., Olbing, H.G., Spitzer, A. and Edelmann, C.M. Neonatal changes in renal blood flow distribution in puppies. Am. J. Physiol. 228: 1453-1461, 1975.

Assali, N.S., Bekey, G.A. and Morrison, L.W. Fetal and neonatal circulation. In Assali, N.S. (ed.) Biology of gestation, Vol. 2, Academic Press, Inc., New York, pp. 51-142, 1968.

Baxter, J.S. and Yoffey, J.M. The postnatal development of renal tubules in the rat. J. Anat. 82:189-196, 1948.

Benyajati, S. and Goldstein, L. Renal glutaminase adaptation and ammonia excretion in infant rats. Am. J. Physiol. 228:693-698, 1975.

Bergeron, M. and Morel, F. Amino acid transport in rat renal tubules. Am. J. Physiol. 216:1139-1149, 1969.

Berndt, W.O. Use of the tissue slice technique for the evaluation of renal transport processes. Environmental Health Perspectives 15:73-88, 1976.

Block, M.B., Pildes, R.S., Mossabhoy, N.A. C-peptide immunoreactivity (CPR): A new method for studying infants of insulin-treated diabetic mothers. Pediatrics 53:923-928, 1974.

Bond, J.T., Bailie, M.D., Narins, D.M. and Hook, J.B. Nutritional factors influencing development of renal organic ion transport. Nutr. Reports Internat. 16: 317-327, 1977.

Bond, J.T., Bailie, M.D. and Hook, J.B. Maturation of renal organic acid transport in vivo: substrate stimulation by penicillin. J. Pharmacol. Exp. Ther. 199:25-31, 1976.

Bradley, S.E., Laragh, J.H., Wheeler, H.O., MacDowell, M. and Oliver, J. Correlation of structure and function in the handling of glucose by the nephrons of the canine kidney. J. Clin. Invest. 40:1113-1131, 1961.

Brasel, J. and Winick, M. Maternal nutrition and prenatal growth. Arch. Dis. Child. 47:479-485, 1972.

Braunlich, J. The renal excretion of inulin and para-aminohippuric acid in rats of different ages. Acta. Biol. Med. Ger. 24:317-335, 1970.

Brodehl, J. and Gellissen, K. Endogenous renal transport of free amino acids in infancy and childhood. Pediatrics 42:395-404, 1968.

Calcagno, P.L. and Rubin, M.I. Renal extraction of para-aminohippurate in infants and children. J. Clin. Invest. 42:1632-1639, 1963.

Cartier, P., Belanger, P., Lemieux, G. Characteristics of *in vitro* ammonia and glucose production by dog kidney cortex. Am. J. Physiol. 228:934-943, 1975.

Cassady, G., Hinkley, C., Bailey, P., Blake, M. and Younger, B. Ammiotic fluid creatinine in pregnancy complicated by diabetes. Am. J. Obstet. Gynecol. 122:13-20, 1975.

Cheek, D.B., Maddison, T.G., Malinek, M. and Coldbeck, J.H. Further observations on the corrected bromide space of the neonate and investigation of water and electrolyte status in infants born of diabetic mothers. Pediatrics 28:861-869, 1961.

Cheek, D.B., Brayton, J.B. and Scott, R.E. Overnutrition, overgrowth, and hormones (with special reference to the infant born of the diabetic mother). Adv. Exp. Med. Biol. 49:47-72, 1974.

Cook, C.D., O'Brien, D., Hansen, J.D.L., Beem, M. and Smith, C.A. Water and electrolyte economy in newborn infants of diabetic mothers. Acta. Paediatrica 49: 121-128, 1960.

Cross, R.J. and Taggart, J.V. Renal tubular transport: Accumulation of p-aminohippurate by rabbit kidney slices. Amer. J. Physiol. 161:181-190, 1950.

Davis, B.B., Walter, M.J. and Murdaugh, H.V., Jr. Renal response to graded saline challenge. Am. J. Physiol. 217:1604-1607, 1969.

Dulin, W.E. and Wyse, B.M. Reversal of streptozotocin diabetes with nicotinamide. Proc. Soc. Exp. Biol. 130:992-994, 1969.

Ecker, J.L. and Hook, J.B. Analysis of factors influencing the *in vitro* developmental pattern on p-aminohippurate transport by rat kidney. Biochim. Biophys. Acta 339:210-217, 1974.

Edelmann, C.M., Barnett, H.L., and Troupkou, V. Renal concentrating mechanisms in newborn infants. Effect of dietary protein and water content, role of urea, and responsiveness to antidiuretic hormone. J. Clin. Invest. 39:1062-1069, 1960.

Edelmann, C.M., Jr. and Barnett, H.L. Role of the kidney in water metabolism in young infants. J. Pediat. 56:154-179, 1960.

Edelmann, C.M., Soriano, J.R., Boichis, H., Gruskin, A.B., and Acosta, M.I. Renal bicarbonate reabsorption and hydrogen ion excretion in normal infants. J. Clin. Invest. 46:1309-1317, 1967.

Edelmann, C.M., Jr. and Wolfish, N.M. Dietary influences on renal maturation in premature infants. Pediat. Res. 2:421, 1968.

Edelmann, C.M., Jr. and Spitzer, A. The maturing kidney. A modern view of well-balanced infants with imbalanced nephrons. J. Pediat. 75:509-519, 1969.

Falk, G. Maturation of renal function in infant rats. Am. J. Physiol. 181:157-170, 1955.

Farah, A. and Rennick, B.R. Studies on the renal tubular transport of tetraethylammonium ion in renal slices of the dog. J. Pharmacol. 117:478-487, 1956.

Farah, A., Frazer, M. and Porter, E. Studies of the uptake of N-methylnicotinamide by renal slices of the dog. J. Pharmacol. 126:202-211, 1959.

Farquhar, J.W. Metabolic changes in the infant of the diabetic mother. Pediatric Clinics of North America 12:743-764, 1954.

Farquhar, J.W. The infant of the diabetic mother. Clin. Endocrinol. Metabol. 5:237-263, 1976.

Fee, B.A. and Weil, W.B., Jr. The body composition of infants of diabetic mothers by direct analysis. Ann. N.Y. Acad. Sci. 110:869-897, 1963.

Felig, P. Body fuel metabolism and diabetes mellitus in pregnancy. Medical Clinics of North America 61:43-66, 1977.

Fetterman, G.H., Shuplock, N.A., Philipp, F.S. and Gregg, H.S. The growth and maturation of human glomeruli and proximal convolutions from term to adulthood: studies in microdissection. Pediatrics 35:601-619, 1965.

Forrest, J.N. and Stanier, M.W. Kidney composition and renal concentrating ability in young rabbits. J. Physiol. 187:1-4, 1966.

Foulkes, E.C. Effects of heavy metals on renal aspartate transport and the nature of solute movement in kidney cortex slices. Biochim. Biophys. Acta. 241:815-822, 1971.

Fraser, H.S. and Alleyne, G.A.O. The effect of malnutrition on the pattern of growth in the rat kidney and the renal response to acidosis. Br. J. Nutr. 31: 113-124, 1974.

Games, P.A. An improved-t-table for simultaneous control on g contrasts. J. American Statistical Assoc. 72: 531-537, 1977.

Goldstein, L. Relation of glutamate to ammonia production in the rat kidney. Am. J. Physiol. 210:661-666, 1966.

Goldstein, L. Renal ammonia and acid excretion in infant rats. Am. J. Physiol. 218:1394-1398, 1970.

Goldstein, L. Ammonia metabolism in kidneys of suckling rats. Am. J. Physiol. 220:213-217, 1971.

Goldstein, L. and Harley-DeWitt, S. Renal gluconeogenesis and mitochondrial NAD^+/NADH ratios in nursing and adult rats. Am. J. Physiol. 224:752-757, 1973.

Goldstein, R., Hook, J.B. and Bond, J.T. The effects of maternal protein deprivation on renal development and function in neonatal rats. J. Nutr. 109:947-957, 1979.

Goodman, A.D., Fuisz, R.E. and Cahill, G.F., Jr. Renal gluconeogenesis in acidosis, alkalosis and potassium deficiency: Its possible role in regulation of renal ammonia production. J. Clin. Invest. 45:612-619, 1966.

Gruskin, A.B., Edelmann, C.M. and Yuan, S. Maturational changes in renal blood flow in piglets. Pediat. Res. 4:7-13, 1970.

Gumbrecht, J., Hook, J.B., Bailie, M.D. and Bond, J.T. Effects of prenatal over-nutrition on renal development in the rat. Nutr. Reports Internat. 16:329-337, 1977.

Hall, S.M. and Zeman, F.J. Kidney function of the progeny of rats fed a low protein diet. J. Nutr. 95:49-54, 1968.

Hauser, C.A. and Barley, E. Changes in the activities of kidney gluconeogenesis during development of the rat. Int. J. Biochem. 6:61-64, 1975.

Heller, H. The renal function of newborn infants. J. Physiol. 102:429-440, 1944.

Heller, H. The response of newborn rats to the administration of water by the stomach. J. Physiol. 106: 245-255, 1947.

Heller, H. The action and fate of vasopressin in newborn and infant rats. J. Endocrinol. 8:214-223, 1952.

Hems, D.A. Biochemical aspects of renal ammonia formation in metabolic acidosis. Enzyme 20:359-380, 1975.

Hirsch, G.H. and Hook, J.B. Maturation of renal organic acid transport. Substrate stimulation by penicillin. Science 165:909-910, 1969.

Hirsch, G.H. and Hook, J.B. Maturation of renal organic acid transport: substrate stimulation by penicillin and PAH. J. Pharmacol. Exp. Ther. 171:103-108, 1970(a).

Hirsch, G.H. and Hook, J.B. Stimulation of renal organic acid transport and protein synthesis by penicillin. J. Pharmacol. Exp. Ther. 174:152-158, 1970(b).

Hirsch, G.H., Cowan, D.F. and Hook, J.B. Histological changes in normal and drug-induced development of renal PAH transport. Proc. Soc. Exp. Biol. and Med. 137:116-121, 1971.

Hook, J.B., Williamson, H.E. and Hirsch, G.H. Functional maturation of renal PAH transport in the dog. Can. J. Physiol. Pharmacol. 48:169-175, 1970.

Hook, J.B. Postnatal development of renal transport systems in the maturing kidney. Proc. 5th Int. Congr. Nephrol. 1:6-13, 1974.

Hook, J.B. and Hewitt, W.R. Development of mechanisms for drug excretion. Am. J. Med. 62:497-506, 1977.

Horster, M. and Lewy, J.E. Filtration fraction and extraction of PAH during neonatal period in the rat. Am. J. Physiol. 219:1061-1065, 1970.

Horster, M. and Valtin, H. Postnatal development of renal function. Micropuncture and clearance studies in the dog. J. Clin. Invest. 60:779-795, 1971.

Hoy, P.A. Diuresis in newborn rats given intravenous water or salt solution. Proc. Soc. Exp. Biol. Med. 122: 358-361, 1966.

Janicki, R.H. and Goldstein, L. Glutamine synthetase and renal ammonia metabolism. Am. J. of Physiol. 216: 1107-1110, 1969.

Johnston, D.I. and Bloom, S.R. Neonatal glucagon response in infants of diabetic mothers. In. Camerini-Davalos, R.A., and Cole, H.S. (eds) Early diabetes in early life. Academic Press, Inc., New York, pp. 541-546, 1975.

Jose, P.A., Logan, A.G., Slotkolf, L.M., Lilienfield, L.S., Calcagno, P.L. and Eisner, G.M. Intrarenal blood flow distribution in canine puppies. Pediat. Res. 5: 335-345, 1971.

Junod, A., Lambert, A.E., Orci, L., Pictet, R., Gonet, A.E. and Renold, A.E. Studies on the diabetogenic action of streptozotocin. Proc. Soc. Exp. Biol. Med. 126: 201-205, 1967.

Kaplan, A. Urea nitrogen and urinary ammonia. In Meites, S. (ed.) Standard methods of clinical chemistry. Academic Press, Inc., New York, pp. 245-246, 1965.

Kaplan, M.R. and Lewy, J.E. Stimulation of p-aminohippurate extraction in the maturing rabbit kidney. Pediat. Res. 12:834-837, 1978.

Kervran, A., Guillaume, M. and Jost, A. The endocrine pancreas of the fetus from diabetic pregnant rat. Diabetologia 15:387-393, 1978.

Kim, J.K., Hirsch, G.H. and Hook, J.B. In vitro analysis of organic ion transport in renal cortext of the newborn rat. Pediat. Res. 6:600-605, 1972.

Kitzmiller, J.L., Cloherty, J.P., Younger, M.D., Tabataball, A., Rothchild, S.B., Sosenko, I., Epstein, M.F., Singh, S. and Neff, R.K. Diabetic pregnancy and perinatal morbidity. Am. J. Obstet. Gynecol. 131: 560-580, 1978.

Kittelson, J.A. The postnatal growth of the kidney of the albino rat with observations on an adult human kidney. Anat. Rec. 13:385, 1917.

Kleinman, L.I. and Hsieh, N.N. Renal tubular response of the newborn dog to a sodium load. Pediat. Res. 8: 456, 1974.

Kleinman, L.I. and Reuter, J.H. Maturation of glomerular blood flow distribution in the newborn dog. J. Physiol. 228:91-99, 1973.

Kolinska, J. Kinetics of sugar transport in rabbit kidney cortex, in vitro movement of D-galactose, 2-deoxy-D-galactose, and methyl-D-glucoside. Biochim. Biophys. Acta. 219:200-209, 1970.

Krebs, H.A., Bennett, D.A.H., DeGasquet, P., Gascoyne, T. and Yoshida, T. Renal gluconeogenesis. Biochem. J. 86:22-27, 1963.

Lassiter, W.E., Gottschalk, C.W. and Mylle, M. Micropuncture study of net transtubular movement of water and urea in non-diuretic mammalian kidney. Am. J. Physiol. 200:1139-1146, 1961.

Lassiter, W.E., Mylle, M., and Gottschalk, C.W. Net transtubular movement of water and urea in saline diuresis. Am. J. Physiol. 206:669-673, 1964.

Leake, R.D., Trystad, C.W. and Oh, W. Inulin clearance in the newborn infant: Relationship to gestational and postnatal age. Pediat. Res. 10:759-762, 1976.

Ljundqvist, A. Fetal and postnatal development of intrarenal arterial pattern in man. Acta Paediatr. Scand. 52:443-464, 1963.

Loggie, J.M.H., Kleinman, L.I. and Van Maanen, E.F. Renal function and diuretic therapy in infants and children. Part I. J. Pediat. 86:485-496, 1975.

Lowery, G.H., Graham, B.D. and Tsao, M.U. Chemical homeostasis in the newborn IDM. Pediatrics 13:527-535, 1954.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193:265-275, 1951.

McCance, R.A. and Wilkinson, E. The response of adult and suckling rats to the administration of water and hypertonic solutions of urea and salt. J. Physiol. 106: 256-263, 1947.

McCance, R.A., Naylor, N.J.B. and Widdowson, E.M. The response of infants to a large dose of water. Arch. Dis. Child. 29:104-109, 1954.

McCance, R.A. and Widdowson, E.M. Hypertonic expansion of the extracellular fluids. Acta Paediatrica 46:337-353, 1957.

McCance, R.A. and Widdowson, E.M. Renal aspects of acid-base control in the newly born. 1. Natural development. Acta Paediatr. Scand. 49:409-414, 1960.

McCance, R.A. Development of acid-base control. Acta. Paediatr. Acad. Sci. Hung. 2:27-31, 1961.

McCance, R.A., Crowne, R.S. and Hall, T.S. The effect of malnutrition and food habits on the concentrating power of the kidney. Clin. Sci. 37:471-490, 1969.

McCrory, W.W. Developmental Nephrology. Harvard University Press, Cambridge, MA, 1972.

Mintz, D.H., Chez, R.A. and Hutchinson, D.L. Subhuman primate pregnancy complicated by streptozotocin-induced diabetes mellitus. J. Clin. Invest. 51: 837-847, 1972.

Mudge, G.H. and Taggart, J.V. Effect of acetate on the renal excretion of p-aminohippurate in the dog. Am. J. Physiol. 161:191-197, 1950.

Naeye, R.L. Fetal lung and kidney maturation in abnormal pregnancies. Arch. Pathol. 99:533-535, 1975.

Naftalin, R.J. A model for sugar transport across red cell membranes without carriers. Biochem. Biophys. Acta. 211:65-78, 1970.

Noordewier, B. and Withrow, C.D. Effects of substrate stimulation on the maturation of PAH transport. Fed. Proc. 35:224, 1976 (Abstract).

Olbing, H., Blaufox, D., Aschingberg, L.C., Silkalns, G.I., Bernstein, J., Spitzer, A. and Edelmann, C.M. Postnatal changes in renal glomerular blood flow distribution in puppies. J. Clin. Invest. 52:2885-2895, 1973.

Osler, M. Renal function in newborn infants of diabetic mothers. Acta. Endocrinol. 34:287-298, 1960.

Osler, M. and Pederson, J. The body composition of newborn infants of diabetic mothers. Pediatrics 26:985-992, 1960.

Pagliara, A.S. and Goodman, A.D. Relationship of renal cortical gluconeogenesis, glutamate content and production of ammonia. J. Clin. Invest. 49:1967-1974, 1970.

Persson, B. Treatment of diabetic pregnancy. In Shafrir (ed.) Contemporary topics in the study of diabetes and metabolic endocrinology. Academic Press, Inc., New York, pp. 83-90, 1975.

Pitkin, R.M., Plank, C.J. and Filer, L.J. Fetal and placental composition in experimental maternal diabetes. Proc. Soc. Exp. Biol. Med. 138:163-166, 1971.

Pitkin, R.M. and Van Orden, O.A. Fetal effects of maternal streptozotocin diabetes. Endocrinology 94:1247-1253, 1974.

Pitts, R.F., Pilkington, L.A., Macleod, M.B. and Leal-Pinto, E. Metabolism of glutamine by the intact functioning kidney of the dog. J. Clin. Invest. 51:557-565, 1972.

Pitts, R.F. Physiology of the Kidney and Body Fluids. 3rd ed., Yearbook Med. Publ. Inc., Chicago, IL 1976.

Potter, E.L. Development of the human glomerulus. Arch. Path. 80:241-255, 1965.

Potter, D., Jarrah, A., Sakai, T., Harrah, J. and Holliday, M.A. Character of function and size in kidneys during normal growth in rats. Pediat. Res. 3:51-59, 1969.

Preuss, H.G. Pyridine nucleotides in renal ammonia metabolism. J. Lab. Clin. Med. 72:379-382, 1968.

Preuss, H.G. Renal glutamate metabolism in acute metabolic acidosis. Nephron 6:235-241, 1969.

Preuss, H.G. and Weiss, F.R. Rate limiting factors in rat kidney slice ammoniagenesis. Am. J. Physiol. 221: 458-464, 1971.

Preuss, H.G., Vavatsi-Manos, O., Vertuno, L. and Baird, K. The effects of pH change on renal ammoniagenesis in vitro. Proc. Soc. Exp. Biol. Med. 146:803-808, 1974.

Preuss, H.G., Eastman, S.T., Vavatsi-Manos, O., Baird, K. and Roxe, D.M. The regulation of renal ammoniagenesis in the rat by extracellular factors. I. The combined effects of acidosis and physiological fuels. Metabolism. 27:1626-1638, 1978(a).

Preuss, H.G., Baird, K. and Eastman, S.T. The regulation of renal ammoniagenesis in the rat by extracellular factors. II. Ammoniagenesis by rat kidney slices incubating in normal acidotic sera. Metabolism 27: 1639-1647, 1978(b).

Prod'hom, L.S., Levison, H., Cherry, R.B., Drorbaugh, J.E., Hubbell, J.P. and Smith, C.A. Adjustment of ventilation, intrapulmonary gas exchange, and acid-base balance during the first day of life. Normal values in well infants of diabetic mothers. Pediatrics 33: 682-693, 1964.

Rakieten, N., Rakeiten, M.L. and Nadkarni, M.V. Studies of the diabetogenic action of streptozotocin. Cancer Chemother. Rep. 29:91-98, 1963.

Rennick, B., Hamilton, B. and Evans, R. Development of renal tubular transports of TEA and PAH in the puppy and piglet. Am. J. Physiol. 201:743-746, 1961.

Rerup, C.C. Drugs producing diabetes through damage of the insulin secreting cells. Pharmacol. Rev. 22:485-518, 1970.

Reynolds, R.A. and Segal, S. Regulatory characteristics of amino acid transport in newborn rat renal cortex cells. Biochim. Biophys. Acta. 426:513-525, 1976.

Reynolds, R., Roth, K.S., Hwang, S.M. and Segal, S. On the development of glycine transport systems by rat renal cortex. Biochim. Biophys. Acta. 511:274-284, 1978.

Robillard, J.E., Sessions, C., Kennedy, R.L. and Smith, F.G. Maturation of the glucose transport process by the fetal kidney. Pediat. Res. 12:680-684, 1978.

Roobol, A. and Alleyne, G.A.O. Control of renal cortex ammoniagenesis and its relationship to renal cortex gluconeogenesis. Biochim. Biophys. Acta. 362:83-91, 1974.

Rosenberg, L.E., Blair, A. and Segal, S. Transport of amino acids by slices of rat kidney cortex. Biochim. Biophys. Acta. 54:479-488, 1961.

Rosenberg, L.E., Downing, S.J., and Segal, S.: Competetive inhibition of dibasic amino acid transport in rat kidney. J. Biol. Chem. 237:2265-2270, 1962.

Roxe, D.M., Schreiner, G.E. and Preuss, H.G. Regulation of renal gluconeogenesis and ammoniagenesis by physiologic fuels. Am. J. Physiol. 225:908-911, 1973.

Scharrer, E., Landes, C. and Scholl, H. Effects of insulin upon renal amino acid transport in lambs. Experientia 30:1416-1418, 1971.

Schrier, R.W. and DeWardener, H.E. Tubular reabsorption of sodium ion: Influence of factors other than aldosterone and glomerular filtration rate. N. Engl. J. Med. 285: 1231 and 1292, 1971.

Schwartz, G.T., Goldsmith, D.I. and Fine, L.G. p-Aminohippurate transport in the proximal straight tubule: Development and substrate stimulation. Pediat. Res. 12:793-796, 1978.

Segal, S., Rea, C. and Smith, I. Separate transport systems for sugars and amino acids in developing rat kidney cortex. Proc. Natl. Acad. Sci. 68:372-376, 1971.

Segal, S., Rosonhagen, M. and Rea, C. Developmental and other characteristics of α-methyl-D-glucoside transport by rat kidney cortex slices. Biochim. Biophys. Acta. 291:519-530, 1973(a).

Segal, S., Genel, M., Holtzapple, P. and Rea, C. Transport of alpha-methyl-D-glucoside by human kidney cortex. Metabolism 22:67-76, 1973(b).

Segal, S., Sutherland, J.M., Lucey, J.F., Drorbaugh, J.E., Cherry, R.B. and Hsia, D.Y. Determination of pH, CO_2 content, O_2 saturation, and lactate in the blood of the newborn infant of diabetic mother. Am. J. Dis. Child. 94:562, 1957.

Siegel, S.R., Fisher, D.A. and Oh, W. Serum aldosterone concentrations related to sodium balance in the newborn infant. Pediatrics 53:410-413, 1974.

Siegel, S.R. and Oh, W. Renal function as a marker of human fetal maturation. Acta. Paediatr. Scand. 65:481-485, 1976.

Silbernagle, S., Foulkes, E.C. and Deetjen, P. Renal transport of amino acids, Rev. Physiol. Biochem. Pharmacol. 74:105-167, 1975.

Silverman, M. Glucose transport in the kidney. Biochim. Biophys. Acta. 457:303-351, 1976.

Sleeper, R.S., Vertuno, L.L., Strauss, F. and Preuss, H.G. Effects of acid challenge on *in vivo* and *in vitro* rat renal ammoniagenesis. Life Sciences 22:1561-1572, 1978.

Smith, H.W., Finkelstein, N., Aliminosa, L., Crawford, B. and Graber, M. The renal clearances of substituted hippuric acid derivatives and other aromatic acids in dog and man. J. Clin. Invest. 24:388-404, 1945.

Smith, E.K., Reardon, H.S. and Field, S.H. Urinary constituents of infants of diabetic and nondiabetic mothers. J. Pediat. 64:652-665, 1964.

Sokol, R.J. and Hall, P.W. Fetal renal tubular function during late pregnancy and diabetes mellitus. Am. J. Obstet. Gynecol. 129:208-210, 1977.

Solomon, F. Embryomegaly and increased fetal mortality in pregnant rats with mild alloxan diabetes. Diabetes: 45-50, 1959.

Solomon, S. and Capek, K. Increased food availability and renal development of neonatal rats. Biol. Neonate 21: 9-15, 1972.

Solomon, S. Developmental changes in nephron number, proximal tubular length and superficial nephron glomerular filtration of rats. J. Physiol. 272:573-589, 1977.

Solomon, S., Bengele, H.H. and Evan, A.P. Amino acid uptake by fetal kidney slices. Biol. Neonat. 29: 257-261, 1976.

Sosenko, I.S., Hartig-Beeken, I. and Frantz, I.D. Glucocorticoid effects on delayed lung maturation in fetuses of alloxan diabetic rabbits. Pediat. Res. 13:506, 1979 (Abstract).

Speller, A.M. and Moffat, D.B. Tubulo-vascular relationship in the developing kidney. J. Anat. 123:487-500, 1977.

Spitzer, A. and Edelmann, C.M. Maturational changes in pressure gradients for glomerular filtration. Am. J. Physiol. 221:1431-1435, 1971.

Stanier, M.W. Development of intra-renal solute gradients in foetal and postnatal life. Pfluegers Arch. 336: 263, 1972.

Stapleton, T. The pattern of the electrolyte excretion in the urine of babies born to diabetic mothers. Arch. Dis. Child. 31:42-43, 1956.

Steel, R.G.D. and Torrie, J.H. Principles and Procedures of Statistics. McGraw-Hill, New York, 1960.

Steinke, J. and Driscoll, S.G. The extractable insulin content of pancreas from fetuses and infants of diabetic and control mothers. Diabetes 14:573-578, 1965.

Stopp, M. and Braunlich, H. Kinetics of p-aminohippurate transport in renal cortical slices from neonatal and adult rats. Biochemical Pharmacology 26:1809-1812, 1977.

Svenningsen, N.W. Renal acid-base titration studies in infants with and without metabolic acidosis in the postnatal period. Pediat. Res. 8:659-672, 1974.

Szalay, J. and Gaal, M. Clinical and morphological studies in streptozotocin diabetic pregnant rats. Acta. Medica Academiae Scientiarum Hungariae 32:35-41, 1975.

Tannen, R.L. Relationship of renal ammonia production and potassium homeostasis. Kidney Internatl. 11:453-465, 1977.

Tsang, R.C., Kleinman, L.I., Sutherland, J.M. and Light, I.J. Hypocalcemia in infants of diabetic mothers. J. Pediat. 80:384-395, 1972.

Tsang, R.C., Chen, F., Friedman, M.A., Gigger, M., Steichen, J., Koffler, H., Fenton, L., Brown, D., Pramanik, A., Keenan, W., Strub, R. and Joyce, T. Parathyroid function in infants of diabetic mothers. J. Pediat. 86: 399-404, 1975.

Turner, R.J. and Silverman, M. Sugar uptake into brush-border vesicles from normal human kidneys. Proc. Natl. Acad. Sci. 74:2825-2829, 1977.

Tudvad, F. Sugar reabsorption in premature and full term babies. Scand. J. Clin. Lab. Invest. 1:281-283, 1949.

Van Marthens, E., Grauel, L. and Zamenhoff, S. Enhancement of prenatal development of operative restriction of litter size in the rabbit. Life Sciences 11:1031-1035, 1972.

Venkatachalam, P.S. and Ramanathan, K.S. Effect of protein deficiency during gestation and lactation on body weight and composition of offspring. J. Nutr. 84: 38-42, 1964.

Wachstein, M. and Bradshaw, M. Histochemical localization of enzyme activity in the kidneys of three mammalian species during their postnatal development. J. Histochem. Cytochem. 13:44-56, 1965.

Wacker, G.R., Zarkowsky, H.S. and Burch, H.B. Changes in kidney enzymes of rats after birth. Am. J. Physiol. 200:367-369, 1961.

Webber, W.A. and Cairns, J.A. A comparison of the amino acid concentrating ability of the kidney cortex of newborn and mature rats. Can. J. Phsyiol. Pharmacol. 46:165-169, 1968.

Webber, W.A. A comparison of the efflux rates of AIB from the kidney cortex slices of mature and newborn rats. Can. J. Physiol. Pharmacol. 46:765-769, 1968.

Weil, W.B., Jr. Evaluation of renal function in infancy and childhood. Am. J. Med. Sci. 229:678-694, 1955.

Weldon, V.V., Kowarski, A. and Migeon, C.J. Aldosterone secretion rates in normal subjects from infancy to adulthood. Pediatrics 39:713-723, 1967.

Winick, M. and Noble, A. Cellular response in rats during malnutrition at various ages. J. Nutr. 89:300-306, 1966.

Yaffe, S.J. and Anders, T.F. Renal solute content in young rabbits. Am. J. Dis. Child. 100:358-359, 1960.

Zeman, F.J. Effects of maternal protein restriction on the kidney of the newborn young of rats. J. Nutr. 94: 111-116, 1968.

Zetterstrom, R. and Aberg, B. Infants of diabetic mothers. II. Studies on the electrolyte metabolism and the effects of starvation during the first days of life. Acta. Paediatrica 44:1-16, 1955.

Zetterstrom, R. and Arnhold, R.C. Impaired calcium-phosphate homeostasis in newborn infants of diabetic mothers. Acta. Paediatrica 47:107-112, 1958.

Zorzoli, A., Turkenkopf, I.J. and Mueller, V.L. Gluconeogenesis in developing rat kidney cortex. Biochem. J. 11:181-185, 1969.