Renal Development in High-Glucose Ambience and Diabetic Embryopathy

By Sumant S. Chugh, Elisabeth I. Wallner, and Yashpal S. Kanwar

Maternal diabetes has an adverse influence on the intrauterine growth of the fetus, which is attributable to the exposure of the mammalian embryo to an abnormal metabolic environment. A sustained exposure of the fetus to such an environment (ie, elevated concentration of glucose), during the first 6 to 8 weeks of gestation in humans may result in diabetic embryopathy, which is characterized by a multitude of congenital birth defects, including those of the nervous, cardiovascular, skeletal, and urogenital systems. The urogenital abnormalities may be associated with caudal regression syndrome or may occur alone in the form of partial or total renal agenesis. Similarly, an increase in the incidence of morphogenetic defects is observed in offsprings of streptozotocin-induced diabetic rats and mice and also in nonobese diabetic mice. In certain instances, failure in the growth of lower part of embryos or newborn mice has been observed in animals with a severe diabetic state. For further delineation of the mechanisms involved in the pathogenesis of diabetic embryopathy, the investigators used whole-embryo culture systems, and found that glucose can induce defects mainly confined to the lower part of the body involving the genitourinary system. Similarly, dysmorphogenesis of the embryonic metanephros is observed when it is subjected to high concentrations of D-glucose and its epimer D-mannose. This article discusses certain aspects of diabetic embryopathy with an emphasis on changes that occur in the fetal metanephros in high-glucose ambience.

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DIABETES MELLITUS is a metabolic disorder in which hyperglycemia inflicts acute reversible and chronic cumulative irreversible damage in several organ systems, including blood vessels, nerves, muscles, eye, and kidney.1,2 The acute metabolic changes may be caused by increased activity of the polyol pathway, perturbations in myoinositol and diacylglycerol levels, activation of protein kinase C, glycation of proteins, and generation of oxygen free radicals that ultimately inflict damage to tissues. The chronic changes are seen in molecules with a relatively slow metabolic turnover (eg, DNA, eye lens protein, and collagen). These changes are irreversible and are related to the persistent long-term hyperglycemia, as a consequence of which some of the extracellular matrix (ECM) proteins (eg, collagen) undergo nonenzymatic glycation. The glycated collagen leads to an abnormal cross-linking with other proteins with excessive accumulation and disordered organization of the ECM basement membranes, a hallmark finding described as a renovascular complication of diabetes mellitus in adult life.3

WHAT IS DIABETIC EMBRYOPATHY?

The complications seen during embryonic life can be described as diabetic embryopathy, meaning thereby that there is an abnormal development of the embryo in a maternal hyperglycemic environment. Similar to the diverse complications in adult life, the development of multiple organ systems is affected during embryonic life, and the malformations induced collectively are referred to as diabetic embryopathy (DE). The concept of DE as described in the past literature includes a 2- to 4-fold increased incidence of malformations occurring during embryogenesis in offsprings of diabetic mothers between the 3rd and 7th week (ie, at the end of blastogenesis and at the beginning of organogenesis).4-6 DE is always under consideration whenever a pregnant woman has a history of pregestational diabetes mellitus.7 The factors influencing the outcome of DE include insulinopenia, severity and duration of diabetes mellitus and its therapeutic control, and the age of diabetic mothers. The malformations seen in DE often are confined to the central nervous, cardiovascular, gastrointestinal, genitourinary, and musculoskeletal systems (Table 1).8,9 The malformations vary in extent and severity, and all of them may not occur in a given fetus or its organ system, suggesting a lack of specificity of the assault by hyperglycemia.
on the embryo. The malformations of the musculoskeletal system may include agenesis of the lumbosacral spine, sirenomelia, or underdevelopment of the lower extremities associated with major visceral abnormalities, and they are referred to collectively as caudal regression syndrome (Fig 1).\(^9\)

The visceral abnormalities may include malformation of the genitourinary system, which can occur in the absence of caudal regression syndrome. Among the various malformations, the incidence of caudal regression syndrome was reported to be the highest in older literature.\(^5\) However, subsequent studies indicate that the incidence of cardiac anomalies, including the minor ones, may be the highest;\(^11\) this possibly may be related to the improved detection of defects by newer techniques (eg, sonography, and so forth). Nonetheless, caudal regression syndrome remains the most noteworthy abnormality that is associated with aberrant urogenital development. The malformations also are seen in embryos or newborn rats of mice with a streptozotocin-induced diabetic state. Interestingly, in our studies we noted that it is the lower part of the body that mostly is affected by the malformations in such embryos/fetuses as well.

### POTENTIAL PATHOGENETIC MECHANISMS OF DE

In most likelihood, the pathogenesis of DE is multifactorial,\(^12,13\) and it would include oxidant stress, perturbed biosynthesis of prostaglandins and DNA, and altered expression of some of the morphogenetic regulatory molecules, including ECM proteins, transcription factors, and proto-oncogenes. The perturbation in some of these molecules may be interdependent. For instance, reactive oxygen species (ROS) can directly damage the DNA and also alter the expression of some of the ECM macromolecules, suggesting that oxidant stress may be the common denominator in the pathogenesis of diabetic embryopathy as is the case with other complications of diabetes mellitus in adult life.\(^14,15\)

### Oxidant Stress in DE

Interest in the role of oxidant stress in the pathogenesis of diabetes arose from the studies from the mid-1970s when a remarkable decrease in the reduced-glutathione (GSH) levels was observed in erythrocytes of the STZ-induced diabetic rats.\(^16\) With the continued interest, the studies of the early 1980s indicated that there are decreased levels of

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**Table 1. Malformations in Offsprings of Diabetic Mothers**

<table>
<thead>
<tr>
<th>Organ System</th>
<th>Description of Malformations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central nervous</td>
<td>Open neural tube defects, abnormal growth of brain, absent corpus callosum, Arnold-Chiari</td>
</tr>
<tr>
<td></td>
<td>anomaly, holoprosencephaly, micro- and macro-ccephaly, schizencephaly, hydrocephaly and</td>
</tr>
<tr>
<td></td>
<td>agenesis of olfactory tract</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>Transposition of large blood vessels, tetralogy of Fallot, coarctation of aorta, cardiac</td>
</tr>
<tr>
<td></td>
<td>hypoplasia, atrial and ventricular septal defects</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>Pyloric stenosis, duodenal and anorectal atresia, hypoplastic colon and omphalo-enteric</td>
</tr>
<tr>
<td></td>
<td>cysts</td>
</tr>
<tr>
<td>Urogenital</td>
<td>Renal agenesis, uterine agenesis, hypoplastic testes, penis and vagina, cryptorchidism,</td>
</tr>
<tr>
<td></td>
<td>ureterocele, duplication of ureter, hydronephrosis and renal cysts</td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td>Caudal regression syndrome, craniosynostosis and abnormalities of lower extremities</td>
</tr>
<tr>
<td>Others</td>
<td>Situs inversus, brachial arch defects of thymus, thyroid and parathyroid, cranio-facial</td>
</tr>
<tr>
<td></td>
<td>abnormalities</td>
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</table>
cysteine-insensitive (manganese-containing superoxide dismutase) in various tissues of diabetic animals,\textsuperscript{17-19} and this was followed by a flurry of activity in this field in the 1990s. One of the recent findings that strengthened the original observation for the role of oxidant stress in the pathogenesis of diabetic complications relates to F\textsubscript{2}-isoprostane. Increased levels of F\textsubscript{2}-isoprostane, indicative of lipid peroxidation and ROS generation, were detected in diabetic rats, and interestingly, the levels were normalized by vitamin E (\(\alpha\)-tocopherol) treatment.\textsuperscript{20} Currently, ROS are regarded as the common denominator and pivotal to the development of diabetic complications in adult life.\textsuperscript{14,15} However, their direct role in DE is somewhat debatable, although there are some correlative studies that support their role in the pathogenesis of embryonic dysmorphogenesis in a hyperglycemic environment. Eriksson et al performed the seminal studies to delineate the relationship between ROS and diabetic embryopathy.\textsuperscript{8} In the early 1990s they observed that the frequency and degree of embryonic malformations, induced either by high glucose, 3-hydroxybutyrate, or \(\alpha\)-ketoisocaproic acid, were reduced by addition of ROS scavengers (ie, manganese-containing superoxide dismutase, catalase, glutathione peroxidase, and N-acetylcysteine) in the culture medium.\textsuperscript{21,22} Later, N-acetylcysteine was shown to normalize the glucose-inhibited migration and proliferation of embryo-derived neuroectodermal cells.\textsuperscript{23} Besides these inhibitory studies, an alternative way to address the issue of ROS would be to determine the activity of oxygen radical scavengers or tissue levels of oxidants and antioxidants. In this regard, embryos of diabetic rats and those subjected to a high-glucose environment have low levels of \(\alpha\)-tocopherol, GSH, and GSH synthesizing enzyme, \(\gamma\)-glutamylcysteine synthetase.\textsuperscript{24-26} They also have increased concentration of intra- as well as extracellular free oxygen radicals in isolated embryonic cells exposed to high glucose levels.\textsuperscript{27} Additional support for the glucose-induced ROS-mediated injury is derived from the fact that a relatively low catalase activity is observed in embryos of rats that have the propensity to develop congenital malformations in offspring.\textsuperscript{28} Finally, analogous to the observations in adult animals, some of the in vivo studies have reported an increase in the tissue levels of F\textsubscript{2}-isoprostane in midgestational embryos of diabetic rats, although other studies have shown that administration of vitamin E (\(\alpha\)-tocopherol) to pregnant rats reduces the incidence of malformations in the offsprings.\textsuperscript{26}

**Perturbation in Arachidonic Acid and Prostaglandin E\textsubscript{2} Metabolism**

Studies from the 1990s have documented a decreased concentration of prostaglandin E\textsubscript{2} (PGE\textsubscript{2}) in the yolk sac of embryos of diabetic women and in the murine diabetic embryos at the time of neural tube closure,\textsuperscript{29,30} suggesting that its deficiency may play a role in congenital malformations. In addition, down-regulation of cyclooxygenase-2 (COX-2), an inducible form of COX that converts arachidonic acid (AA) to prostaglandins, as well as of PGE\textsubscript{2}, has been observed in embryos cultured under high-glucose conditions.\textsuperscript{31} Further support for this notion is derived from the studies that showed an accentuated uptake of AA by the embryonic yolk sacs in hyperglycemic states.\textsuperscript{32} Direct evidence as to the role of AA was obtained from studies in which the extent of neural tube defects were reduced by the in vivo administration of AA, a precursor of PGE\textsubscript{2}, to the pregnant diabetic animals.\textsuperscript{33} Also, addition of AA or PGE\textsubscript{2} to the culture medium has been shown to block the high-glucose- or diabetic serum–induced dysmorphogenesis in the murine embryos.\textsuperscript{34,35}

A complementary, but somewhat surprising, finding pertinent to AA-, COX-2–, or PGE\textsubscript{2}-mediated effects is that glucose-induced embryonic dysmorphogenesis can be reciprocated by COX inhibitors,\textsuperscript{34} and the development of such lesions is blocked with the supplementation of the culture medium with AA or PGE\textsubscript{2}. Intriguingly, COX inhibitor–induced dysmorphogenesis in the embryos could be reduced notably by the antioxidant therapy, such as by addition of superoxide dismutase and N-acetylcysteine in the culture medium.\textsuperscript{34} This suggests that perhaps there is a cross-communication between the 2 biologic systems that exerts their interdependent effect in the induction of congenital malformations in diabetic embryos. The antioxidant treatment restores the PGE\textsubscript{2} concentration, however, the COX-2 levels are unaffected. Conceivably, the oxidant stress in diabetes reduces the enzyme activity of COX-2 by lowering the availability of its cofactor, GSH, which suggests that COX-2 inhibition may be the primary event that contributes to embryopathic lesions in diabetes.
Perturbation of Macromolecules With Slow Metabolic Turnover

Potentially, the molecules that may play an essential role in embryonic morphogenesis and could be damaged by the hyperglycemic state includes DNA and ECM morphogenetic proteins. Conceivably, DNA can be damaged by the free oxygen radicals; which may be reflected by the breaks in the DNA strands or mutations, the latter situation arises when there is a failure in the recognition of homologous bases in DNA segments by the reparative enzymes. Similar damage to DNA has been shown to occur in hyperglycemic states; and by exposure to reducing sugars, such as D-glucose- or D-fructose-6-phosphate.\textsuperscript{36,37} Incubation of DNA with these reducing sugars perhaps leads to accelerated unwinding of its strands followed by scission in a time- and concentration-dependent manner, leading ultimately to DNA mutations. Such glucose-induced oxidant stress damage is reported to be preventable in embryos by the administration of vitamin E to diabetic mothers.\textsuperscript{38} To address the issue of frequency of DNA mutations in a diabetic environment, Lee et al developed a transgenic mouse model system in which mutation frequency was evaluated in the neutral target reporter gene, \textit{lacI}, in the embryos that were transferred to normal and pseudopregnant diabetic recipient mice.\textsuperscript{39} An increased rate of DNA mutations was observed in the reporter gene in embryos implanted into a diabetic environment. The results of these in vivo observations were confirmed by the in vitro findings, in which increased DNA mutations were observed in midgestational embryos that were cultured in high-glucose conditions. Other molecules in a hyperglycemic environment that may induce DNA damage include methyl glyoxal and 3-deoxyglucosone, a powerful glycating agent. The tissue concentrations of the latter have been reported to be increased in diabetic embryos and the DNA damage during this carbonyl stress most likely is mediated by the superoxide ion.\textsuperscript{40}

The ECM proteins are another group of molecules that similar to DNA are germane to the pathobiology of DE. The ECM proteins were the first macromolecules that were regarded as crucial to the embryonic development because they modulate epithelial-mesenchymal interactions that are prevalent during embryogenesis.\textsuperscript{41} Various ECM morphogenetic proteins include proteoglycans, fibronectin, laminin, and collagens. Their role in the branching morphogenesis of salivary glands, breast, prostate, lung, and kidney has been well documented.\textsuperscript{32,43} As an inference of their role in morphogenesis or organogenesis, one can anticipate their relevance in the pathogenesis of diabetic complications, including diabetic embryopathy and nephropathy. In support of the latter, a number of studies performed during the 1980s and 1990s have reported alterations in gene and protein expression of ECM proteoglycans, collagens, and fibronectin.\textsuperscript{44,45} Similar alterations in the ECM proteins have been reported to occur in the embryos that are developing in a diabetic environment.\textsuperscript{45} An increase in the expression of laminin and fibronectin was observed in day 11 and 12 embryos and in the heart and kidney of the newborn fetuses of diabetic mice. Such altered ECM gene expression, in particular of the fibronectin, was observed in day 9 embryos exposed to a high-glucose environment.\textsuperscript{45} Interestingly, altered fibronectin expression in the rats also is seen during hyperglycemia in the organ that is shared by the mother and fetus (ie, the placenta). Similar changes have been reported in human term placenta of diabetic mothers,\textsuperscript{46} suggesting a materno-fetal commonality in the development of diabetic complications.

Another set of macromolecules that may be of crucial relevance but understudied in the pathogenesis of DE are proto-oncogenes. Their role in embryonic organogenesis using transgenic techniques has been elucidated in mice.\textsuperscript{47} Also, there are few reports that describe their status in the complications of diabetes in adult life (eg, an increased expression of \textit{c-jun}, \textit{c-fos}, and \textit{c-myc} have been reported in diabetic nephropathy).\textsuperscript{48,49} Equally few are the literature reports that deal with the subject of proto-oncogenes in diabetic embryopathy. Recently, the status of \textit{Pax-3} was described and its decreased expression was correlated with the neural tube defects.\textsuperscript{50} Finally, there are ongoing further studies to search for new gene(s) that may be relevant to the pathogenetic mechanism(s) of DE (eg, the Dep-1 gene).\textsuperscript{51}

**EFFECT OF HYPERGLYCEMIA/HIGH-GLUCOSE AMBIENCE ON THE EMBRYONIC DEVELOPMENT OF MAMMALIAN KIDNEY**

Very few studies have been reported in the literature that describe the development of individual
organ systems during hyperglycemia or in a high-glucose environment despite the fact that the developmental events can be recapitulated readily in culture systems of many organs (eg, the kidney, lung, prostate, and pancreas). Most of the studies reported pertain to the pathobiology of the kidney. The impetus for renal studies stems from the fact that diabetic nephropathy has been the subject of intense investigation for more than 4 decades. Another reason may be that kidney abnormalities do occur in offspring of diabetic mothers and sometimes are associated with the caudal regression syndrome.

General Features of Metanephric Development

Renal development ensues after the successive appearance of the pronephros, mesonephros, and metanephros as a cranio-caudal wave of cellular differentiation in the nephrogenic cord lying alongside the nephric or Wolffian duct. In mammals, the pronephros and mesonephros are vestigial elements of the nephrogenic cord, and their appearance is transitory, whereas the metanephros matures to form a permanent kidney. Metanephrogenesis commences at about day 11 of fetal life in the mouse and at about the 5th week of gestation in humans by sequential and reciprocal inductive interaction between the mesenchyme and the ureteric bud. During this process the ureteric bud, an epithelial-lined tubular structure arising from the Wolffian duct, interacts with the blastema, a loosely organized mesenchymal mass on the lateral aspect of the aorta in the most caudal segment of the nephrogenic cord. The interaction leads to differentiation of the mesenchyme into an epithelial phenotype, reciprocal inductive arborization of the ureteric bud, and generation of nascent nephrons. After inductive epithelial-mesenchymal interaction, the nascent nephrons are formed by undergoing the following developmental stages: condensate-vesicle, comma-shaped body, S-shaped, and precapillary bodies. The precapillary bodies on vascularization by the processes of vasculogenesis and angiogenesis form functioning mature glomeruli of the kidney.

Further insights into the developmental dynamics of the metanephros were obtained by the usage of an in vitro culture organ culture system established by Grobstein. In this culture system, various developmental stages, with the exception of vascularization of the metanephros, can be studied. The technique uses harvesting of either uninduced (day 10.5) or induced (day 11.5) metanephric mesenchyme, placing it on a microporous filter and maintaining it in culture for 7 to 10 days. The induced mesenchyme, as in vivo, undergoes a series of differentiation events leading to glomerulo- and tubulogenesis, whereas the uninduced mesenchyme may undergo apoptosis, and part of it develops into stroma or interstitium of the kidney. Finally, fundamental to the inductive neotransformation of the mesenchyme are the molecules expressed at the epithelial-mesenchymal interface, or ligands expressed in the mesenchyme, whereas receptors are expressed in the ureteric bud epithelium. It should be noted that, at times, receptors may be expressed in the mesenchyme whereas ligands are expressed in the ureteric bud epithelium.
kidney. Interdependence between growth factors and ECM proteins is reflected by the fact that the latter may act as storage depots for certain growth factors or may contain growth factor–like domains. In such a scenario, the ECM would be expected to modulate the biologic activities of various growth factors (eg, transforming growth factor β), while transforming growth factor β in turn regulates the synthesis of ECM proteins. Similarly, c-ret, a proto-oncogene that regulates nephrogenesis and ECM expression in metanephric tissues, serves as a receptor for glial cell line–derived nerve growth factor. The latter is related structurally to the transforming growth factor β superfamily and plays a vital role in embryonic organ development. Thus, the biology relative to renal development of this diverse group of molecules, including ECM proteins, integrin receptors, growth factors and their receptors, proto-oncogenes, and other putative molecules, is linked intricately and has been reviewed recently.

Aldohexose(s) Induced Dysmorphogenesis of the Embryonic Kidney

During the past decade, D-glucose– and D-mannose–induced dysmorphogenesis of the metanephric kidneys, using a transfilter organ culture system, have been described. The impetus for these studies came from the observations of caudal regression syndrome in which, as indicated earlier, that urogenital system often is affected. Second, the entire urogenital system has been found to be rudimentary in 3% to 5% of newborn mice with poor development of the lower somites. Also, day 13 embryos affected by hyperglycemic injury showed poor development of the caudal portion of the body, and the embryonic metanephiroi are difficult to delineate. In view of the earlier-described inherent difficulties, the organ culture system has been used to study the effect of D-glucose or its epimer, D-mannose, on the developing embryonic murine metanephiroi, and diverse morphologic and biochemical changes have been described.

Morphologic Changes

The changes induced by D-glucose or D-mannose are similar, except the extent of dysmorphogenesis was relatively severe with the latter when accounting for their treatment on the basis of mole to mole ratios. Overall, the size of the metanephros is reduced considerably after an exposure of 30 mmol/L D-glucose for 1 week. The ureteric bud itself is swollen and its iterations are thickened. Their tips usually are blunted and deformed. These tips of the ureteric bud branching are the site for epithelial-mesenchymal interactions that are necessary for nascent nephron genesis. Interestingly, the radio-incorporation of various markers for protein and proteoglycan synthesis is reduced concomitantly. The proteoglycans are known morphogenetic modulators, therefore their deficiency would be expected to lead to dysmorphogenesis of the metanephros and reduced population of the nephrons. Also, further contribution to the reduction in the population is related to the fulminant apoptosis. The apoptosis of the mesenchymal cells at the interface especially would be expected to interfere in the epithelial-mesenchymal interactions with consequential reduction in the nascent nephron concentration. It should be noted that basal apoptosis is a normal occurrence during nephrogenesis, however, it is highly accentuated in metanephiroi exposed to high-glucose ambience.

Besides the overall reduction in size of the metanephros and branching dysmorphogenesis of the ureteric bud, the population of nascent nephrons, including S-shaped bodies and precapillary stage, are reduced substantially after 7 days of exposure to D-glucose/mannose. The reduction in the population of nephrons is discernible even after 48 hours of treatment. Notable are the changes in the S-shaped body, especially its distal portion, which gets deformed with reduced radio-incorporation of sulfated proteoglycans. The distal limb of the S-shaped body is a progenitor of proximal, distal, and collecting tubules, whereas the proximal limb forms precapillary and capillary structures of the glomerulus. On closer examination of the D-glucose/mannose-treated metanephiroi, one observes that the tubulogenesis is relatively much more severely affected compared with glomerulogenesis, suggesting that aldohexoses have a pronounced deleterious effect on tubular development. Interestingly, a similar phenotype is seen with the gene disruption of tubulointerstitial nephritis antigen (TINag) in vitro. TINag is an ECM protein expressed exclusively in the tubules, and has been shown to modulate tubulogenesis in vitro culture systems. This would suggest that D-glucose adversely affects the expression of TINag, which would be correlative of the phenotype observed in
high-glucose ambience. Studies are ongoing in our laboratory to investigate the biochemical changes that may occur in TINag expression owing to hyperglycemic injury, and to see whether TINag is involved in epithelial-mesenchymal interactions during mammalian metanephric development.

Another set of unique molecules that are ideally suited for epithelial-mesenchymal interactions include imprinted genes that are expressed strategically in mammalian metanephros and are affected by high-glucose ambience injury.72 The imprinted genes studied so far in the metanephros include H19 and mesodermal-specific complementary DNA, the latter also known as MEST. MEST is expressed in the mesenchyme and is imprinted paternally, whereas H19 is expressed in the ureteric bud branches and is expressed maternally. Both of them exhibit stage-specific messenger RNA expression (ie, they are highly expressed during midgestation and disappear a few days after birth in neonatal mice). Having such a stage-specific and spatiotemporal expression would suggest that they have an important role in mammalian metanephric development73; which conceivably could be compromised in a hyperglycemic injury. Indeed, the exposure of D-glucose tremendously reduces their messenger RNA expression in both the metanephric mesenchyme as well as in the ureteric bud branches. The MEST expression was reduced at a relatively earlier time frame compared with that of H19 in the embryos of diabetic mice, which would go along with the fact that metanephric mesenchyme is much more susceptible to high-glucose ambience injury, as corroborated by fulminant apoptosis observed in the mesenchyme.69 There are various other imprinted genes (eg, IGF-1 and IGF-1R) that exhibit spatiotemporal expression in metanephros and their status in a hyperglycemic injury to the developing kidneys needs to be investigated.

Biochemical Changes

The major biochemical changes studied pertain to the ECM morphogenetic modulators of development, including sulfated proteoglycans.68,69 Changes in other ECM proteins (eg, type IV collagen and lamin-A chain) do occur during D-glucose–induced dysmorphogenesis of the kidney, but the most striking alterations are seen in heparan sulfate proteoglycan (HS-PG). The changes in HS-PG expression are accompanied by a significant size reduction of its protein as assessed by gel filtration chromatography. In addition, alterations in the posttranslational modifications also are observed, and they include decreased sulfation of the HS-PG, which goes along with the in vivo studies in which reduced sulfation is observed in hepatocytes in a diabetic state.74 In addition to HS-PGs, changes in the de novo synthesis of other PGs (eg, chondroitin sulfate-proteoglycan) has been described.70 The mechanism involved in the reduction may include oxidant stress, in which ROS can damage the integrity of the PGs, similar to other ECM glycoproteins. The fulminant apoptosis observed in D-glucose–treated metanephros would suggest that ROS played a role in the altered expression of the PGs. Interestingly, the reduction is especially notable at the epithelial-mesenchymal interface while at the same region the apoptosis is observed maximally, suggesting that the interaction between different cellular elements is the key event leading to dysmorphogenesis of the murine metanephros.

Another mechanism by which the HS-PG expression is affected relates to the changes in its transcription. Structural analysis of the 5′ end and upstream region of the HS-PG (perlecan) has revealed the presence of 2 viral enhancer activated protein-2 (AP-2) motifs and 3 small palindromic repeats, which by forming secondary structures may regulate the expression of HS-PG.75 AP-2 mediates transcriptional activation either via cyclic adenosine monophosphate–dependent protein kinase A or via phorbol-ester– and diacylglycerol–activated protein kinase C.76 The protein kinase C is distributed ubiquitously in various tissues, and it has been reported to alter the gene expression of certain ECM proteins (eg, laminin, type IV collagen, and fibronectin).77 Presumably, protein kinase C–induced fibronectin expression is mediated by the transcription factor AP-1.78 The role of AP-1 in the expression of ECM proteins remains to be investigated. In regard to AP-2, a reduction in its activity in metanephroi treated with D-glucose has been observed, suggesting that glucose either reduced the intracellular concentration of AP-2 or its active moiety. The latter possibility is likely because insulin normalizes the DNA–protein interactions, conceivably by phosphorylation of the transcription factor AP-2.69

The earlier-described observations indicate that glucose may perturb various metabolic events that are dependent on intracellular phosphorylation or...
D-glucose–induced dysmorphogenesis has been observed in a novel system designed by Abrass et al and is comparable with in vivo conditions. They used ocular engraftment of the embryonic kidney in syngeneic animals having a hyperglycemic state. A delay in the rate of glomerular maturation associated decreased expression of laminin-β2, a known morphogen, was observed. The earlier-described studies, albeit very few, support the contention that D-glucose and its epimers (ie, mannose and galactose) can indeed induce dysmorphogenesis of kidney, conceivably by altering the juxtacrine-paracrine interactions of the molecules that are expressed in the metanephric mesenchyme and the ureteric bud epithelia. Further studies certainly are warranted to understand the mechanism(s) that are involved in the dysmorphogenesis of the kidney as well as of the organ systems during hyperglycemic states to delineate a common etiologic denominator that also is relevant to the pathogenesis of diabetic embryopathy and dysmorphogenesis of newborns of diabetic women. J Reprod Med 7:61-64, 1971


energy stores (ie, adenosine triphosphate [ATP] level). In this regard, other aldohexoses (eg, mannose) have been shown to induce depletion of ATP levels. The decrease in the ATP level may lead to a reduced phosphorylation of AP-2, and consequential reduced gene expression of PGs. It also is conceivable that decreased expression of HS-PGs may be affected directly by the depletion of ATP stores because the PGs are glycosylated heavily and require substantial amounts of ATP for their synthesis and posttranslational modifications, including sulfation. In support for this contention are studies in which exogenous addition of ATP into the medium normalizes the [35 S]-sulfate incorporation, proteoglycan expression, and morphology of the murine metanephros treated with D-glucose.

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