Histol Histopathol (1995) 10: 979-993

Histology and Histopathology

Invited Review

The glucose-6-phosphatase system in human development

A. Burchell[1] and R. Hume[2]

Departments of [1]Obstetrics and Gynaecology and [2]Child Health and Biochemical Medicine, Ninewells Hospital and Medical School, University of Dundee, Dundee, Scotland

Summary. The classical role of glucose-6-phosphatase in liver and kidney is the production of glucose for release into blood. In liver, glucose-6-phosphatase catalyses the terminal step of glycogenolysis and gluconeogenesis. Abnormally low hepatic glucose-6-phosphatase activity is found in human genetic deficiencies i.e. glycogen storage disease type I and in cases of developmental delay, found predominantly in preterm infants. In contrast, abnormally high liver glucose-6-phosphatase occurs in poorly controlled or untreated diabetes mellitus.

Hepatic glucose-6-phosphatase is an integral endoplasmic reticulum (and nuclear membrane) protein and it is part of a multicomponent system. Its active site is situated inside the lumen of the endoplasmic reticulum and transport proteins are needed to allow its substrates glucose-6-phosphate (and pyrophosphate) and its products phosphate and glucose to cross the endoplasmic reticulum membrane. In addition, a calcium binding protein is also associated with the glucose-6-phosphatase enzyme. Immunohistochemical studies, in combination with image analysis, have shown that glucose-6-phosphatase is present in liver and kidney and also in specific cell types in a variety of human tissues, for example Leydig cells in the testis and some astrocytes in the brain. Where practicable, enzymatic analysis, direct transport assays and/or immunological detection of the endoplasmic reticulum glucose and phosphate transport proteins have been used to demonstrate the presence and activity of the whole glucose-6-phosphatase system. The distribution of the human glucose-6-phosphatase system changes dramatically during development with a different spatial and temporal pattern in each tissue. The most unexpected localization was in circulating, predominantly nucleated, embryonic and early fetal red blood cells.

Offprint requests to: Dr. Ann Burchell, Department of Obstetrics and Gynaecology, University of Dundee, Ninewells Hospital and Medical School, Dundee, DD1 9SY, Scotland, UK

Key words: Glucose-6-phosphatase, Endoplasmic reticulum, Image analysis, Development

Introduction

Most tissues cannot make sufficient amounts of glucose to maintain their metabolic functions and need a continuous supply from the bloodstream. Dietary supplies of glucose in man are intermittent and to maintain blood glucose levels between meals, the body needs the capability to make glucose. The liver is the major site of glucose production. It plays a major role in regulation of blood glucose levels (Nordlie, 1985) and in response to stress or low blood glucose levels it releases glucose for use by other tissues. The two pathways by which the liver can make glucose are by glycogenolysis and gluconeogenesis (Nordlie, 1976, 1985). One enzyme, microsomal glucose-6-phosphatase (EC 3.1.3.9) catalyses the terminal step of both pathways (Ashmore and Weber, 1959; Burchell, 1992). The hepatic microsomal glucose-6-phosphatase system has been extensively characterised in the last few years (Burchell et al., 1994) and for normal glucose-6-phosphatase activity *in vivo*, several different protein components are needed. The catalytic subunit of the glucose-6-phosphatase enzyme is situated with its active site inside the lumen of the endoplasmic reticulum and it is associated with a regulatory Ca^{2+} binding protein (Fig. 1). Three transport systems termed T1, T2 and T3 (GLUT 7) (Fig. 1) are also needed to respectively transport the substrate glucose-6-phosphate (Fulceri et al., 1992) and the products phosphate (and the substrate pyrophosphate) (Arion et al., 1976, 1980) and glucose across the liver endoplasmic reticulum membrane (Waddell et al., 1991, 1992; Nordlie et al., 1992). *In vitro*, the glucose-6-phosphatase enzyme has been shown to hydrolyse a number of substrates and catalyse a wide range of phospho-transferase reactions (Nordlie, 1976) although the physiological significance of this has not

been unequivocally demonstrated.

Glucose-6-phosphatase is located in the endoplasmic reticulum and until recently has been resistant to protein purification which delayed monospecific antibody production and immunohistochemical methods (Burchell, 1992). In the absence of specific antibodies for immunodetection, demonstration of glucose-6-phosphatase activity has been confined to histochemical and cytochemical techniques based on the lead salt method (Chiquione, 1953; Wachstein and Meisel, 1956). Although the conventional lead salt method has been improved (Wachstein and Meisel, 1956; Maly and Sasse, 1983), concerns about diffusion of reaction products (Essner, 1973), inhibition by lead ions (Marchesi and Palade, 1967), and formation of precipitate (Chiquione, 1953) has led to the development of alternative histochemical methods based on cobalt (Thiery et al., 1990) and cerium (Jonges et al., 1990) for detection. However, non-specific hydrolysis of glucose-6-phosphatase, other than that catalysed by glucose-6-phosphatase, remains a problem in tissue assays of enzyme activity (Burchell and Waddell, 1991). In addition, conditions of tissue preparation prior to histochemistry are exacting (Kanamura, 1971; Berteloot and Hugan, 1975; Brenner et al., 1979; Kalicharan et al., 1985) limiting applications in the main to laboratory animals and exceptionally to human tissue (Benkoel et al., 1989). The availability of monospecific antiserum to the glucose-6-phosphatase enzyme, T2ß and T3, and immunohistochemistry linked to image analysis methods, allows an approach to unequivocally determine the cellular localisation of the enzyme in developing human tissues for the first time, which avoids many of the difficulties inherent in histochemical methods (Table 1).

In addition, the availability of micro-methods for both enzymatic analysis of the glucose-6-phosphatase system (Burchell et al., 1988b) and microsomal transport assays (Waddell et al., 1989; Fulceri et al., 1992) makes it possible for the first time not only to unequivocally determine if glucose-6-phosphatase is present in a particular tissue, but also, to directly analyse individually the glucose-6-phosphatase enzyme and its associate transport proteins during human development (Table 1).

This review will describe the ontogenic changes in expression of the glucose-6-phosphatase system in human tissues in the embryo (up to 56 postovulatory days), in the fetus (8 weeks gestation to term), postnatally (after term birth) and adulthood.

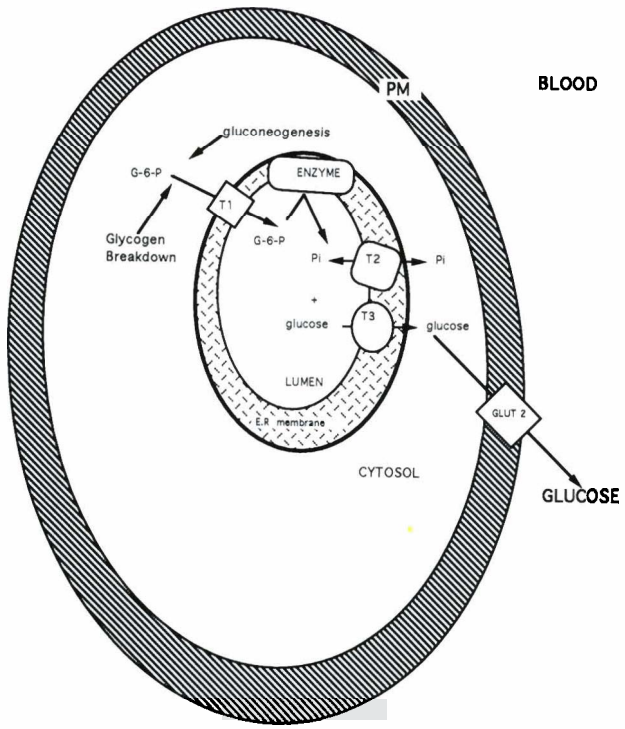


Fig. 1. Schematic representation of the pathways of hepatic glucose production. G-6-P: glucose-6-phosphate; PM: plasma membrane; ENZYME: glucose-6-phosphatase enzyme; T2: endoplasmic reticulum phosphate transport protein; T3: endoplasmic reticulum facilitative glucose transport protein; GLUT 2: plasma membrane facilitative glucose transport protein 2; ER: endoplasmic reticulum.

Liver

The hydrolysis of glucose-6-phosphate by glucose-6-phosphatase in liver was first described in 1945 (Fantl and Rome, 1945). The importance of glucose-6-phosphatase in the regulation of blood glucose levels first became obvious in 1952 from the debilitating effects of the complete absence of the enzyme in type Ia glycogen storage disease (Cori and Cori, 1952; Chen and Burchell, 1995). Deficiencies of other components of the glucose-6-phosphatase system (Fig. 1) also result in type I glycogen storage disease (Burchell, 1992; Burchell and Waddell, 1990, 1993; Burchell et al., 1994). Many of the clinical features of the type I glycogen storage diseases can be related to a liver and or kidney deficiency but others cannot (Chen and Burchell, 1995) suggesting that glucose-6-phosphatase may normally be present in additional tissues. Significant levels of glucose-6-phosphatase were found in human liver and kidney in the 1950's, (see Ashmore and Weber, 1959 for an early review), in keeping with the enzyme's key role in gluconeogenesis. While the enzyme has been extensively studied in adult liver from a wide variety of species, little is known about this system in early human development. In many small laboratory animals, glucose-6-phosphatase enzyme activity increases immediately after birth (e.g. Weber and Cantero, 1955a; Burchell and Leakey, 1988) and the lack of glucose-6-phosphatase in the liver of these in-utero animals has restricted studies in fetal development. However, we have previously shown that glucose-6-phosphatase activity is present in human fetal liver from 12 weeks gestation to term (Burchell et al., 1990) and that the

Table 1. Human tissues in which expression of specific microsomal glucose-6-phosphatase enzyme activity and/or protein and/or mRNA have been reported.

TISSUE	PREDOMINANT DISTRIBUTION	DEVELOPMENTAL STAGE	REFERENCES
Liver	Hepatocytes	Fetal Adult	Cori and Cori, 1952 Hefferan and Howell, 1977 Burchell et al., 1988a, 1990 Chamlian et al., 1991 Hume and Burchell, 1993
Kidney	Proximal tubules Collecting ducts Loop of Henle Distal tubules	Embryonic Fetal Adult	Hume et al., 1994 Burchell et al., 1994
Adrenal	Fetal zone	Fetal	Hume et al., 1995b,e
Gallbladder	Mucosa	Adult	Hill et al., 1989
Brain	Some but not all astrocytes	Fetal Adult	Bell et al., 1993
Intestine	Mucosa	Adult	Ockerman, 1964 Pears et al., 1992
Red blood cells	Nucleated red Blood cells	Embryonic Fetal	Hume et al., 1995c,d
Trachea	Epithelium	Fetal	Burchell et al., 1994 Hume and Burchell, 1995
Oesophagus	Epithelium	Fetal	Burchell et al., 1994 Hume and Burchell, 1995
Testis	Leydig cells	Fetal	Burchell and Hume, 1995

ontogenic pattern is very different from the development in rat (Burchell and Leakey, 1988). Clearly, conventional biochemical studies during the first 12 weeks of in-utero development are restricted by limitations of amounts of tissue available and immunohistochemical methods with image analysis are a logical way of overcoming this problem.

The parenchymal cells of the liver are derived from the hepatic diverticulum of the embryonic foregut and from as early as 26 postovulatory days (Stage 12), columnar cords of epithelium can be seen growing into the surrounding mesodermal stroma (O'Rahilly and Muller, 1987). In the earliest human embryos available to us, 32 postovulatory days (Stage 14), the columns of epithelia are more obvious but only some of those developing hepatocytes are moderately immunopositive for components of the glucose-6-phosphatase system with red blood cells, predominantly megaloblastic, in the liver sinusoids more intensely stained (Fig. 2A). At about the 35th day of gestation, erythropoiesis begins in the liver and between the 12th and 20th week, erythroid precursors represent approximately 50% of the total nucleated cells of this organ (Thomas and Yoffey, 1964). The human liver is the major site of erythropoiesis until around 24-30 weeks gestation and even at term (40 weeks gestation), red blood cell precursors can still be detected (Oski, 1993), although the major site of haemopoiesis has moved to bone marrow. Immunostaining to the components of the glucose-6-phosphatase system is more intense in those erythroid precursors in human fetal liver than in the hepatocytes (Fig. 2B). Human fetal liver biopsy has been used to diagnose genetic defects of a variety of liver enzymes including glucose-6-phosphatase (e.g. Golbus et al., 1988). At the gestational age such liver biopsies are done, 50% of the tissue can be erythropoietic and, in addition, the biopsy sample may also contain whole blood elements. Our observation that erythroid precursors express the glucose-6-phosphatase system (and other liver enzymes, see below) means that enzyme activities assayed in fetal liver biopsies could potentially give very misleading results; e.g. it is easy to envisage that if a different isoform of the enzyme assayed was present in the erythroid precursors then significant activity could still be found, even though absent from all hepatocytes. Hepatocyte staining shows a characteristic reticular pattern (Fig. 2C), commonly seen with antibodies to proteins of the endoplasmic reticulum (Hume et al., 1995a,b) and with a variability in intensity and distribution which persists into adulthood (Fig. 2C). Human fetal bile ducts are also glucose-6-phosphatase immunopositive (Burchell et al., 1992) and glucose-6-phosphatase enzyme activity has been shown in adult human gallbladder (Hill et al., 1989), epithelia with a common embryological origin to that of hepatocytes. The most unexpected result of our studies in human embryonic and fetal liver was our discovery that glucose-6-phosphatase is present in red blood cells when it has been previously repeatedly shown that glucose-6-phosphatase is not present in adult red blood cells (e.g. Hers and De Duve, 1950).

Red blood cells

Two distinct populations of erythrocytes are present in the vascular spaces of the developing embryo-fetus (Oski, 1993). In the earliest embryos available, the red blood cells are predominantly of the primitive megaloblastic series and predominantly nucleated but numbers decline through late embryonic-early fetal life to approach zero after 12 weeks gestation. In fetuses >12 weeks gestation, the erythrocytes are of the definitive or normoblastic series and these show a reciprocal increase with time. In the embryonic period, the percentage numbers of nucleated and non-nucleated red blood cells in the definitive or normoblastic series are small and similar but through fetal life the number of non-nucleated cells increases to around 95% (Hume et al., 1995c). Although red blood cell haemoglobin will change during further development from HbF to HbA, the morphology of the cells remains normocytic.

In embryos, the majority of red blood cells immunopositive for glucose-6-phosphatase, T2ß and T3 antibody preparations are in the primitive megaloblastic cell series with nucleated cells predominating (Fig. 2J). In some embyros, up to 23% of immunoreactive cells are non-nucleated megaloblastic cell types although more commonly this contribution is much less. In early fetal life, mixed populations of megalo- and normoblastic cells are obvious with cells which are nucleated or non-nucleated and with or without variable immunoreactivity to glucose-6-phosphatase, T2ß and T3 antibody preparations (Fig. 2K). Thereafter, the number of immunopositive cells in the definite normoblastic series decreases such that after 12 weeks gestation it is less than 5% of the total (Fig. 2L). In addition, in RNA isolated from human fetal blood 13-20 weeks gestation, RTPCR using primers and probes specific for the liver glucose-6-phosphatase enzyme, confirmed the presence of glucose-6-phosphatase mRNA (Hume et al., 1995c,d).

The fact that the endoplasmic reticulum glucose-6-phosphatase system, whose major site of expression in adults is liver, is present in human embryonic and fetal red blood cells, particularly nucleated cells, indicated it would be sensible to determine whether these cells also contain other endoplasmic reticulum enzyme systems normally found in adult liver. We therefore studied the expression of other endoplasmic reticulum proteins and found that human embryonic and fetal red blood cells contain UDP-glucuronosyltransferases, cytochrome P450 isozymes, cytochrome P450 reductase and prostaglandin H synthase. In addition, we also found the predominantly cytosolic markers 15-hydroxy-prostaglandin dehydrogenase, prostaglandin E_2 (PGE_2) and 13,14-dihydro-15-keto-PGE_2 (Hume et al., 1995d). The fact that those enzymes are present in erythroid precursors means that ontogenic studies of embryonic-fetal liver expression of enzymes, based solely conventional assays, may have to be reassessed (Simpson et al., 1987).

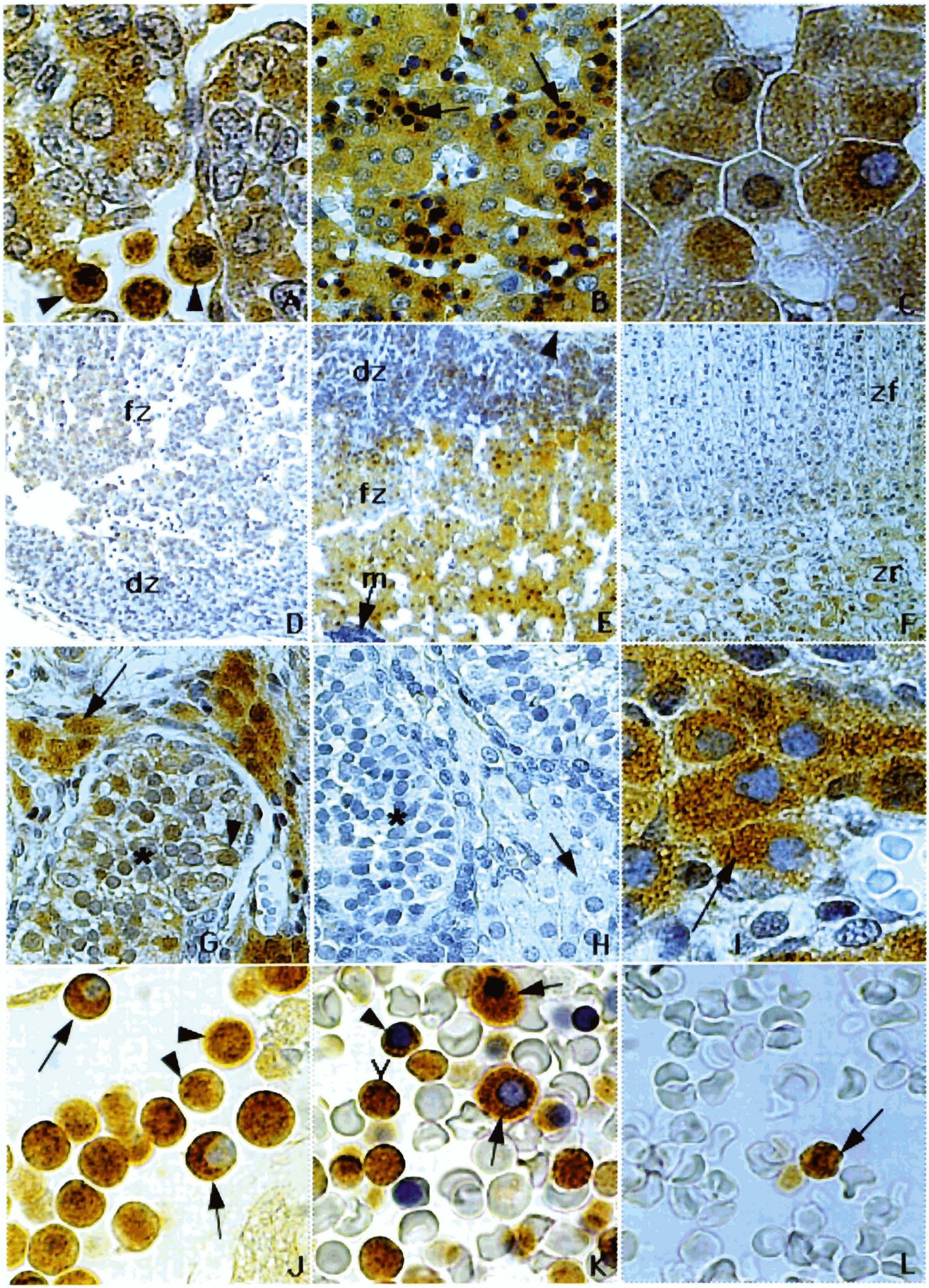
The expression of key enzymes which control glucose production, detoxification of endo- and xenobiotics and the regulation of prostaglandin levels in embryonic and early fetal red blood cells means that these cells may have an important role in protecting the developing conceptus before it establishes an efficient circulation and before all tissues fully express their normal complement of these enzymes.

Kidney

Renal glucose-6-phosphatase activity was first described in the 1950's (Weber and Cantero, 1955b), the specific activity is, surprisingly, higher in kidney than in liver (Weber and Cantero, 1955b). It has also been suggested that in the kidney, glucose-6-phosphatase may play other roles e.g. the regulation of calcium homeostasis in tubular cells (Fulceri et al., 1990) and in the transport of glucose across tubular cells in addition to other established glucose transport mechanisms (Nordlie, 1976).

The kidney glucose-6-phosphatase enzyme and transport protein T2ß show similar patterns with development (Hume et al., 1994, 1995e). In the developing mesonephric kidney of early embryos (32-37 postovulatory days), the most primitive condensations of nephritic tissue, those most caudal, are glucose-6-phosphatase and T2ß immunonegative but, as neprhic vesicles develop progressively rostrally, weak focal glucose-6-phosphatase and T2ß immunopositivity is

Fig. 2. Immunohistochemistry in human tissues. **A.** A 32 postovulatory day liver showing that hepatocytes have variable glucose-6-phosphatase reactivity and that sinusoidal red blood cells are strongly reactive (arrowheads). x 1,140. **B.** A 16 week gestation liver showing moderate glucose-6-phosphatase staining in hepatocytes and intense reactivity in haematopoietic cells (arrows). x 460. **C.** An adult liver showing the variability in intensity and distribution of glucose-6-phosphatase immunoreactivity in hepatocytes. x 1,140. **D.** A 56 postovulatory day embyro showing that T2 immunoreactivity is confined to the fetal zone of the adrenal. x 230. **E.** A 16 week gestation fetus showing that glucose-6-phosphatase immunoreactivity is most intense in the fetal zone, weak in the definitive zone and negative in the medulla. x 115. **F.** An 11 week postnatal infant, born term, showing glucose-6-phosphatase immunoreactive cells in the zona reticularis of the adrenal. x 115. **G.** A 19 week gestation fetal testis showing that glucose-6-phosphatase immunoreactivity is predominantly in Leydig cells (arrow) and in the developing seminiferous tubules (*) where focal cells are positive (arrowhead). x 460. **H.** A 19 week gestation fetal testis (same as A) omitting the glucose-6-phosphatase antibody and showing an absence of immunoreactivity in Leydig cells (arrow) and in the developing seminiferous tubules (*). x 460. **I.** A 16 week gestation fetal testis showing variability in intensity and distribution of glucose-6-phosphatase immunoreactivity in Leydig cells (arrow). x 1,140. **J.** The cardiac chamber of a 50 postovulatory day embryo showing that the majority of blood cells are megaloblastic and variably T2 immunoreactive in nucleated (arrow) and non-nucleated cells (arrowhead). x 1,140. **K.** A 10 week gestation fetal heart showing a mix of megaloblastic and normoblastic blood cells with the majority of T3 immunostaining in nucleated megaloblastic cells (arrow) but also in nucleated (arrowhead) and non-nucleated normoblastic cells (V). x 1,140. **L.** A 16 week gestation fetal aorta showing that the majority of blood cells are normoblastic with only an occasional cell glucose-6-phosphatase immunoreactive (arrow). x 1,140. fz: fetal zone; dz: definitive zone; m: medulla; zf: zona fasciculata; zr: zona reticularis.


A
B
C
fz
dz
D
dz
fz
m
E
zf
zr
F
*
G
*
H
I
J
K
L

present in some cells. With further development of the mesonephric kidney, which is still present up to 9-10 weeks gestation, nephrons, the parietal epithelium of Bowman's capsule and the mesonephric duct are glucose-6-phosphatase and T2ß immunopositive (Fig. 3F) with occasional glomerular podocytes also reactive.

As the ureteric bud of the developing metanephric kidney elongates from 5 to 14 weeks gestation to form the ureter and renal pelvis and then sequentially divides into calyxes and collecting ducts, glucose-6-phosphatase and T2ß immunostaining have a distinct caudal to rostral pattern of reactivity. The epithelium of the ureter and pelvis are strongly immunopositive but this decreases through the length of the developing collecting ducts such that the dividing cells of the ampulla are glucose-6-phosphatase and T2ß immunonegative (Fig. 3G). In early embryos, the cellular distribution of glucose-6-phosphatase and T2ß immunoreactivity are predominantly apical and basal but with further maturation and between 12 weeks gestation and late fetal life, immunoreactivity is displaced predominantly to the apices of cells by perinuclear glycogen deposits (Fig. 3I).

During the period of active nephrogenesis from 8-34 weeks gestation, the subcapsular cells and cellular masses which constitute the metanephric blastema are glucose-6-phosphatase immunonegative (Fig. 3H). With the formation of renal vesicles and S-shaped bodies weak focal positivity is present in some cells and with further development, the parietal layer of Bowman's capsule and developing nephrons become glucose-6-phosphatase and T2ß immunopositive (Fig. 3J). Some glomerular podocytes of the visceral layer of Bowman's capsule are glucose-6-phosphatase immunopositive (Fig. 3J). As nephrons elongate and differentiate from around 12 weeks gestation, all segments (proximal, loop of Henle and distal tubule) retain glucose-6-phosphatase and T2ß immunoreactivity with the most intensive staining in proximal tubules where this has a uniform cellular distribution (Fig. 3J).

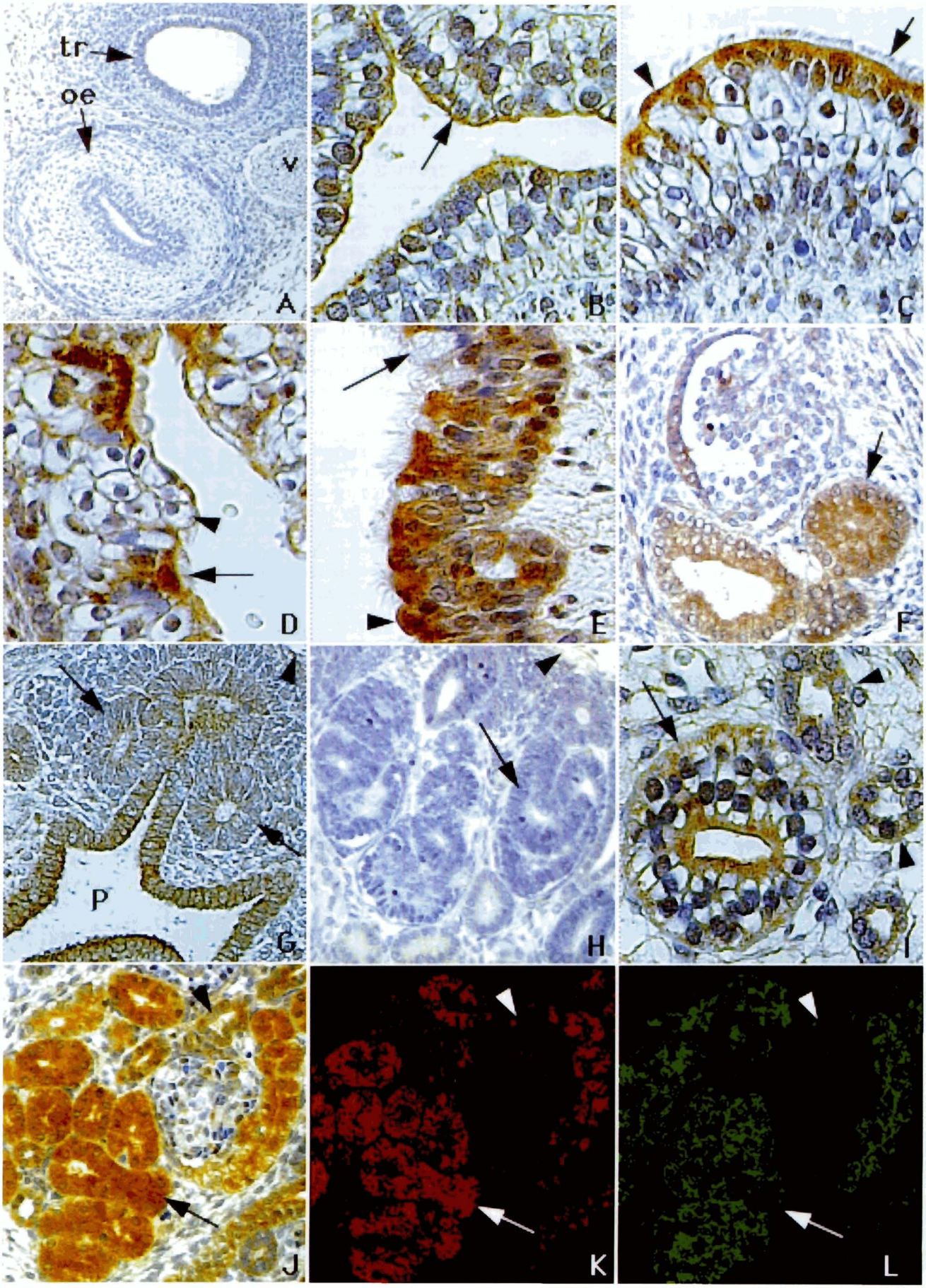
The common spatial and temporal expression of both the T2ß protein and the catalytic unit of the glucose-6-phosphatase system suggests that unlike human fetal liver (Burchell et al., 1990), the kidney T2ß protein is not developmentally delayed compared to the enzyme. Consistent with this is the low latency of glucose-6-phosphatase activity in intact human fetal kidney microsomes with pyrophosphate as substrate, which shows that the level of T2 transport capacity is appropriate for the amount of enzyme activity present (Hume et al., 1995e). This is in complete contrast to the high latency (up to 100%) of glucose-6-phosphatase activity in intact human fetal liver microsomes with pyrophosphate as substrate that we found previously in liver samples of the same gestational range (Burchell et al., 1990; Nordlie et al., 1992). The earlier temporal expression of the endoplasmic reticulum pyrophosphate (and carbamyl phosphate) transport capacity in kidney compared to liver may reflect the importance of pyrophosphate and carbamyl phosphate as substrates in human fetal kidney.

Renal disease is a well recognised late complication of type I glycogen storage disease commonly including focal segmental glomerulosclerosis, interstitial fibrosis, renal stones and nephrocalcinosis and more rarely amyloidosis, a Fanconi-like syndrome and distal renal tubular acidosis (see Chen, 1991; Chen and Burchell, 1995 for reviews). The presentation of overt renal disease in type I glycogen storage disease occurs late in childhood or adulthood and children under 10 years of age usually have normal renal function (Chen et al., 1988), although provocative renal function tests may detect subtle changes (Restaino et al., 1993). We have reported a preterm infant with no T2 pyrophosphate translocase function in either liver or kidney microsomes (Hume et al., 1992). However, it is not known whether this represents either a genetic deficiency affecting both liver and kidney T2 or, whether renal disease in type I glycogen storage disease is a consequence of primary deficiency in kidney or secondary to disregulation of metabolism by the liver defect, or a combination of both.

Image analysis in developing human tissues

Conventional assays of enzyme activities, in for example adult kidney, only gives a result which is the

Fig. 3. Immunohistochemistry in human tissues. **A.** A 47 postovulatory day embryo showing extensive mesenchymal development of trachea (tr, arrow) and oesophagus (oe, arrow), but glucose-6-phosphatase immunonegative epithelium (v, vagus). x 230. **B.** A 10 week gestation fetal oesophagus showing that most apical luminal cells are glucose-6-phosphatase immunopositive (arrow). x 460 **C.** A 14 week gestation fetal oesophagus showing that most apical cells are ciliated (arrow) and glucose-6-phosphatase positive with an occasional reactive non-ciliated cell (arrowhead). x 460. **D.** A 19 week gestation fetal oesophagus showing that ciliated glucose-6-phosphatase reactive cells (arrow) are being replaced by immunonegative squamous epithelium (arrowhead). x 460. **E.** A 16 week gestation fetal trachea showing that ciliated cells, Clara cells (arrowhead), duct lining cells and occasional basal cells are glucose-6-phosphatase immunopositive with mucus secretory cells (arrow) immunonegative. x 460. **F.** A 47 postovulatory day embryonic mesonephric kidney showing that glucose-6-phosphatase reactivity is present in mesonephric tubules (arrow) and parietal layer of Bowman's capsule. x 230. **G.** A 10 week gestation fetal metanephric kidney showing that T2 reactivity is present in the ureteric bud derivatives and this decreases from the pelvis (P) peripherally. Renal capsule (arrowhead), developing renal vesicles (arrows). x 230. **H.** A 16 week gestation fetal kidney showing that the nephrogenic zone and S-shaped bodies (arrow) are glucose-6-phosphatase immunonegative, capsule (arrowhead). x 230. **I.** A 19 week gestation fetal kidney showing that T2 staining is predominantly displaced apically in collecting duct epithelium (arrow) by perinuclear glycogen deposits and that loops of Henle are less reactive (arrowheads). x 460. **J.** A 16 week gestation fetal kidney (same as H) from the midzone showing proximal tubules are intensely glucose-6-phosphatase positive (arrow), distal tubules less reactive (arrowhead) and only occasional cells in the glomerulus are immunopositive x 230. **K.** False colour images of the intensity of glucose-6-phosphatase immunoreactivity in a 16 week gestation fetal kidney (same as J) showing that the most intense staining (false red) is in proximal tubules (arrows) with only foci of reactivity in distal tubules (arrowhead) and in **L)** moderate staining (false green) has a similar pattern of distribution. x 230.


tr
oe
v
A
B
C
D
E
F
P
G
H
I
J
K
L

algebraic sum of activities in the component parts, but no indication as to the relative amounts in different cell types. This is a critical problem when trying to understand the regulation of a key enzyme system, such as the glucose-6-phosphatase system, which has a distribution in multiple cell types within an organ such as kidney, and where the regulators may be cell and or tissue specific. These analytical problems are compounded further during development, when the tissue is rapidly changing in cellular composition and the proportion of cells which express a particular enzyme changes with ontogeny. This is particularly obvious in the developing human metanephric kidney where cells from different mesenchymal origins develop at different rates, i.e. ureteric and nephrogenic zone components, but both express the same enzyme or different isoforms. Described below are our attempts to overcome those problems and allow quantitative immunohistochemistry using image analysis in developing human tissue.

For the acquisition of light microscopic immunohistochemical images we are using a Zeiss microscope - JVC KYF 30-E3 chip colour camera linked to an Apple Macintosh Quadra 950 computer, with a HR-24 RBG card to allow capture of images up to 700 x 752 pixels in size and for control of the high resolution monitor. Colour vision software (Improvision, University of Warwick) is used for video rate capture of 24 bit images with the ability to individually adjust up to five channels to allow thresholding and detection of different coloured populations. For precipitated 3,3'-diaminobenzidine products on immunohistochemical sections we have restricted this to three channels, to dark brown staining (thresholds R1G1B1) monitored as a false red channel, medium brown (thresholds R2G2B2) as a false green channel (see Figs. 3J,K,L) and light brown (thresholds R3G3B3) as a false blue channel, with analysis of the area occupied by each channel per total selected image area. Images and data are routinely stored on optical discs and images captured in Colour vision were transferred through Adobe Photoshop 1.0.7 prior to printing by a Kodak XL-7700 colour printer.

Comparison, by image analysis methods, of an ontogenic series of tissue samples has inherent difficulties. In animal studies, most of the subtle variables in methods, which can occur in the process of fixation and processing, and which can subsequently influence immunohistochemical staining, can be overcome by simultaneous fixation and processing of a developmental series. This cannot be done with a developmental human tissue series, for obvious ethical and technical reasons and the collection of specimens is opportunistic and sporadic and may extend over months

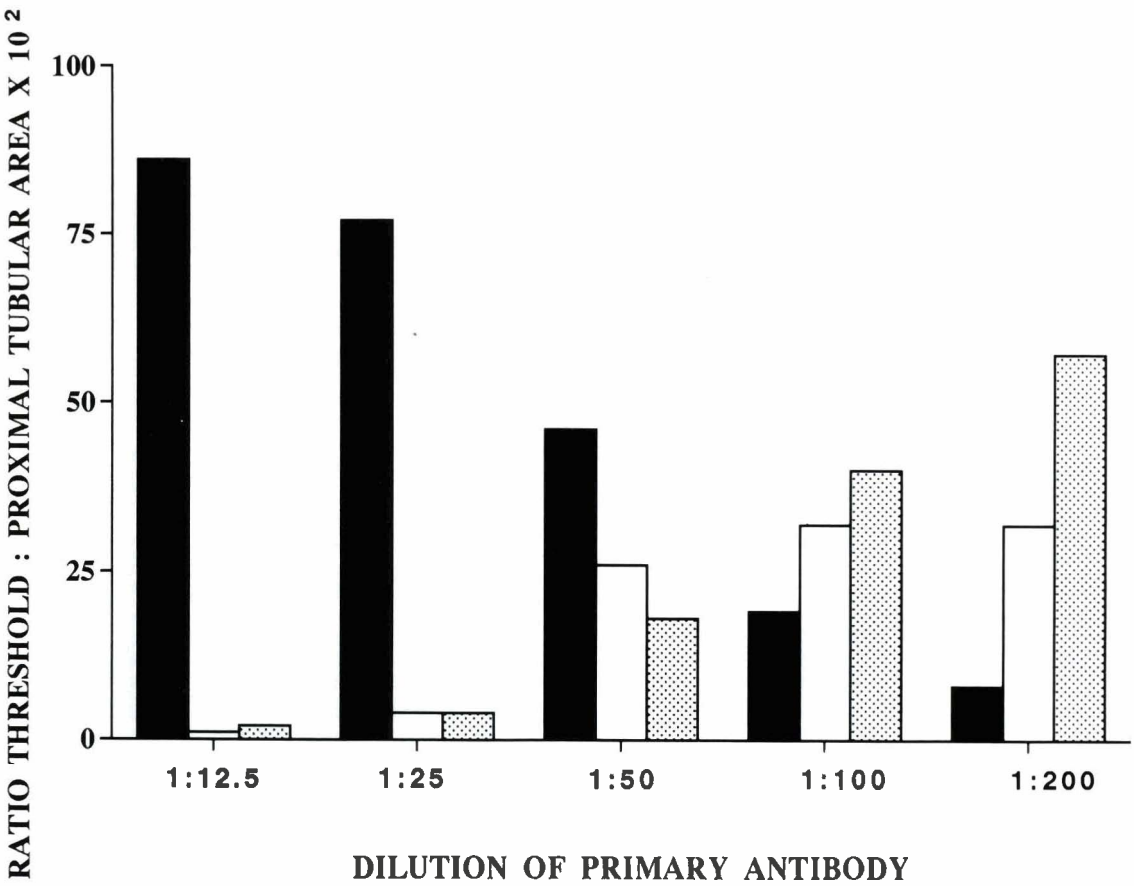


Fig. 4. The ratio of threshold areas (pixel^2) for R1G1B1 (red channel), ■ R2G2B2 (green channel) □ and R3G3B3 (blue channel) ⚄ : total proximal tubular area (pixel^2) x 10^2 for glucose-6-phosphatase immunostaining in a 19 week gestation fetal kidney at various dilutions of the primary antibody.

or years. The tissues in our human developmental series were all fixed (in buffered 10% formalin) and processed in an identical manner over a period of three years, then paraffin blocks sectioned at one session by a skilled operator to achieve uniform thickness of sections.

For studies on developing human kidney, threshold settings for precipitated 3,3'-diaminobenzidine products, R1G1B1 (false red) for dark brown staining, R2G2B2 (false green) for medium brown and R3G3B3 (false blue) for light brown are manually selected using glucose-6-phosphatase or T2ß immunostained proximal tubules in human fetal kidney sections. Proximal tubules are selected for threshold settings as they are the most intensely immunoreactive structures in the fetal and adult human kidney, are easily identified and have a relatively uniformly stained cytosol. The total colour range covered by the threshold settings should allow capture of nearly all of the 3,3'-diaminobenzidine staining in proximal tubules, which is equivalent to approximately 90% of the total epithelial cell area. Individual channels (false red, green and blue) should each represent approximately one third of total 3,3'-diaminobenzidine stainable area (Fig. 4), or if 2 channels are used, (false red and green), each approximately one half of total stainable area (Fig. 3J,K,L) within the range of antibody titres which are considered optimal. The choice of optimal antibody titres in the first instances is designated by semi-quantitative assessment of overall immunostaining reaction in the proximal tubules and the absence of background reactivity in the interstitium of the mesenchyme and glomeruli and confirmed by a lack of reactivity in those areas at the chosen threshold values (Fig. 3J,K,L). All tissue sections from a developmental series are immunostained simultaneously and at two antibody titres around the optimum titre, to allow for minor variations in the staining reaction which can occur between sequential studies of the same series. In the example shown, for glucose-6-phosphatase immunostaining (Fig. 4), the two titres chosen around the optimum are 1:50 and 1:100 with thresholds adjusted for false red, green and blue adjusted such that at both titres sensitivity is retained in all channels. This allows linearity of increments or decrements in immunoreactivity to be maintained and allow quantitation and comparability. In the example shown (Fig. 4), this means that with increasing dilutions of antibody, or decreasing immunoreactivity, the blue channel percentage representing light brown staining with 3,3'-diaminobenzidine increases and conversely with decreasing titres, or increasing immunoreactivity, the red channel percentage representing the darkest staining increases. Once optimal threshold values have been determined for an individual series by manual means, they are then set for automatic thresholding of the subsequent tissue sections to be analysed.

The developmental age of kidney tissue section used to set optimal threshold values is determined by the nature of the process under study, for example, human fetal kidney 16-19 weeks gestation is used as it approximates the mid-point of active nephrogenesis. In addition, the change in many enzyme activities with development often follows the pattern of a sigmoid curve, whether this is increasing or decreasing, and image analysis thresholds are best set at a developmental age which corresponds to the mid-point of the steeply ascending, or descending, portion of the plotted sigmoid curve. In fact we now include tissue sections from a well-characterised 19 week gestation human fetal kidney in each developmental tissue series studied, whether liver or trachea etc, as an internal standard for the immunohistochemical reactions and subsequent image analysis.

As an example of these methods, sequential embryonic and fetal metanephric kidneys sections, were made transversely through the hilum, then immunostained with the T2ß antibody preparation. A series of image sections, 100x572 pixels, were sequentially captured across each of the stained kidney sections from the capsule to the pelvis. Individual image sections were then colour analysed, using the thresholds R1G1B1, R2G2B2 and R3G3B3, and areas (pixel^2) of 3,3'-diaminobenzidine product staining per total image section derived. The individual areas occupied by primitive glomerulo-tubular complexes, glomeruli, proximal tubules, distal tubules, loops of Henle, collecting ducts and papillary-ducts of Bellini epithelium were computed for each serial image and colour analysis of the total area for each component kidney structure from all the serial images was accumulated. A measure of the intensity of T2ß immunostaining in each renal component was derived from the area (pixel^2) of immunostaining divided by the epithelial area (pixel^2) for each component. In the metanephric kidney, before 8 weeks gestation and the onset of nephrogenesis, all glucose-6-phosphatase and T2ß immunostaining is confined to ureteric bud derivatives (Fig. 3G). The localisation of glucose-6-phosphatase and T2ß immunostaining changes with development and analysis of individual serial image sections (100 x 572 pixels) collected sequentially across transverse sections from the hilum to the capsule of three fetal kidneys (16-20 weeks gestation) shows that the majority of T2ß immunostaining is confined to the cortical zone co-localising with proximal tubules, distal tubules and the developing glomerulo-tubular complexes of the nephrogenic zone (Fig. 5). Approximately 30% of T2ß immunostaining is present in the medulla, co-localising with the distribution of collecting ducts and loops of Henle (Fig. 5). The summation of the areas of T2ß immunostaining, equivalent to the false red, green and blue channels, for individual kidney components shows that proximal tubules contribute the greatest epithelial area of immunoreactivity as well as intensity of staining (ratio T2ß immunoreactivity:epithelial area) (Fig. 5). A comparison with glucose-6-phosphatase immunostaining shows a similar pattern of distribution (Hume et al., 1994, 1995b,e).

Adrenal

There have also been several reports of glucose-6-phosphate hydrolysis in adrenal (Hilf et al., 1962; Wegmann and Khosrovchachi, 1964; Meusers, 1966; Jonadet et al., 1968; Colilla et al., 1975) using conventional enzymatic analysis and histochemical methods. Unfortunately all these studies were carried out before it was recognised that glucose-6-phosphatase was a multi-component system, nor was it demonstrated whether the hydrolysis of glucose-6-phosphate seen in adrenal was due to the activity of the glucose-6-phosphatase enzyme or to the action of other non-specific phosphatases e.g. alkaline phosphatase or acid phosphatase which have been demonstrated in many tissues.

As early as 47 postovulatory days, immunoreactivities to the glucose-6-phosphatase, T2ß and T3 antibody preparations are weakly present in the centre of the developing human embryonic adrenal. The structures of the fetal and definitive zones are obvious and distinct by 56 postovulatory days and in addition, moderate immunostaining confined to the fetal zone is present for the three antibody preparations, for example T2 (Fig. 2D). In later gestations, the fetal zone is intensely immunoreactive to the glucose-6-phosphatase, T2ß and T3 antibody preparations (Fig. 2E). The cytoplasmic area of cells in the fetal zone is large and is irregularly

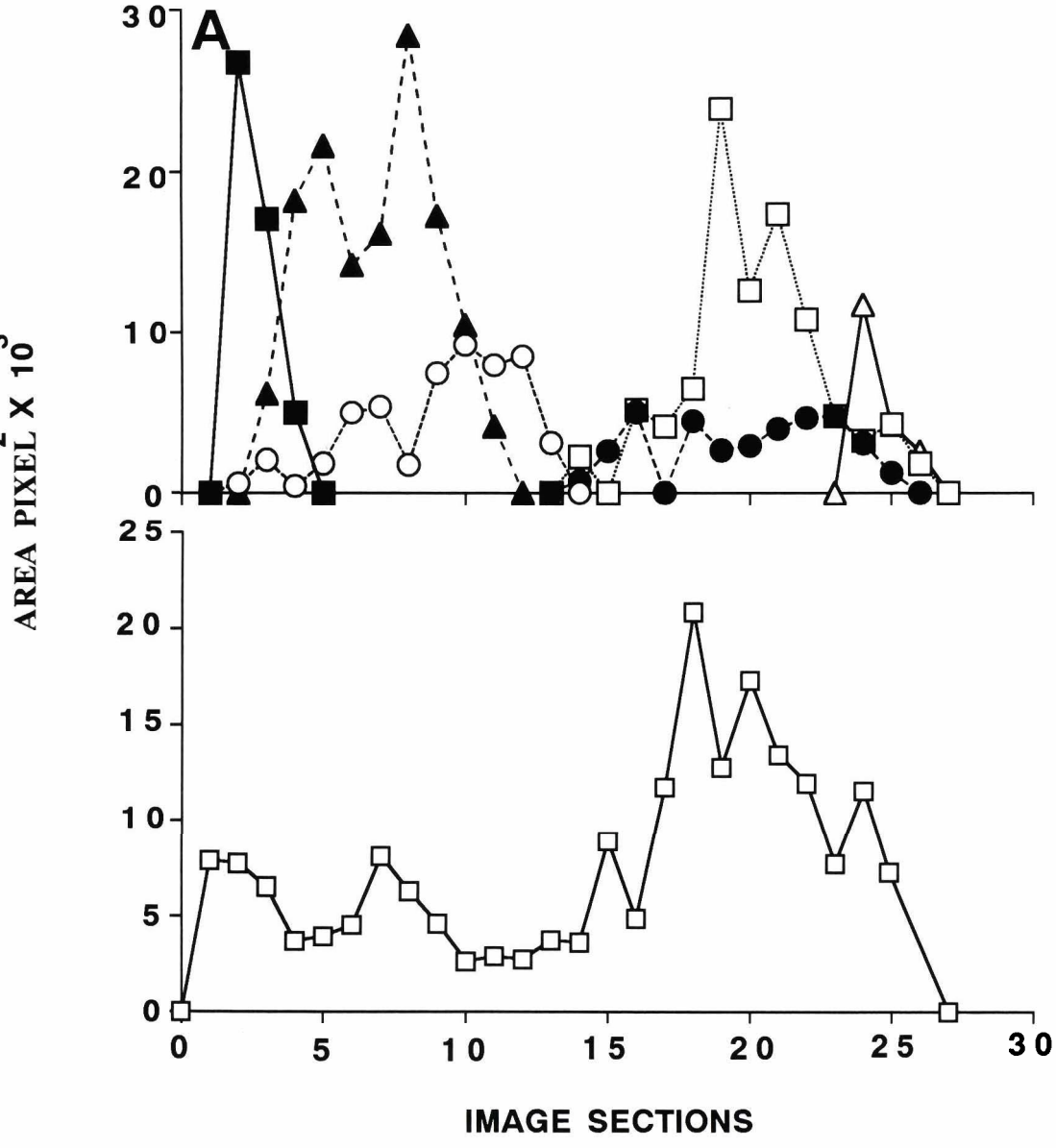


Fig. 5. A. The epithelial areas (pixel^2) of renal components in a series of 100 x 570 pixel transverse image sections from the pelvis (section 0) to the capsule (section 27) in a 16 week gestation human fetal kidney. Proximal tubules (39.5%; 1.0): white squares; distal tubules (10.1%; 0.85%): black circles; primitive glomerulo-tubular complexes (1.4%; 0.22): white triangles; loops of Henle (12.2%; 0.38: white circles; collecting ducts (21.7%; 0.32): black triangles; papillary epithelium-ducts of Bellini (11.4%; 0.50): black squares. The figures in parenthesis are: a) the percentage of total T2ß epithelial immunoreactive area per renal component; and b) the ratio of T2ß immunoreactive area:epithelial area of each component with proximal tubules as nominal value 1.0. **B.** Summation of red and green and blue threshold areas of T2ß epithelial immunostaining (pixel^2) in the same series of 100x570 pixel image sections as A.

immunostained in a reticular pattern with all three antibody preparations, for example glucose-6-phosphatase (Fig. 2E), with marked variability in the intensity and distribution of immunostaining (Fig. 2E; Hume et al., 1995b). The definitive zone appears only weakly immunoreactive, compared to the fetal zone, with all three antibody preparations (Fig. 2E) but at higher magnification a variable number of the small volume definitive zone cells are intensely immunoreactive with complete or partial perinuclear staining (see Hume et al., 1995b). Medullary cells are immunonegative to the glucose-6-phosphatase, T2ß and T3 antibody preparations (Fig. 2E). In postnatal adrenal gland, immunoreactivities to glucose-6-phosphatase, T2ß and T3 are very markedly reduced with only a few large cells in the zona reticularis moderately reactive with faint staining at the junction with the zona fasciculata, for example glucose-6-phosphatase (Fig. 2F).

In addition to the immunohistochemical studies, we have shown specific glucose-6-phosphatase activity and measured the rate of entry of glucose-6-phosphate, phosphate and glucose into microsomes isolated from human fetal adrenals (Hume et al., 1995b). Although the complete enzyme system is present, the ratio of the component activities and comparison to human fetal and adult liver indicates that the regulation of the adrenal and liver glucose-6-phosphatase systems is different. In the human postnatal adrenal, immunoreactivities to the protein components decreases dramatically and is confined predominantly to the zona reticularis, suggesting a specialised role for adrenal glucose-6-phosphatase in fetal life. The role of glucose-6-phosphatase in the adrenal cortex is not clear but its dominance in fetal life may be related to the specific steroidogenic requirements at this time rather than its traditional role in the regulation of blood glucose levels.

Testis

Following on from our observations on adrenal glucose-6-phosphatase, we looked for glucose-6-phosphatase in other steroidogenic organs and found that it was also present in testis (Table 1). There had been several previous reports of glucose-6-phosphate hydrolysis in the adult male reproductive tract using histochemical methods (Vilar et al., 1962; Allen, 1961; Baust et al., 1967; Bogitsh, 1974; Barham et al., 1976; Yohoyama and Chang, 1977; Kanai et al., 1981, 1983, 1986; Hermo et al., 1991; Thorne-Tjomsland et al., 1991) and one using enzymatic analysis (Colilla et al., 1975). But, for the reasons outlined in the Introduction, it is not clear whether they were measuring glucose-6-phosphatase or a non-specific phosphatase. In addition, the reports were conflicting regarding the cellular localisation of glucose-6-phosphate hydrolysis.

Immunoreactivity to glucose-6-phosphatase in the human fetal testis at 12-20 weeks gestation is predominantly found in Leydig cells, with variability in the intensity of staining between cells (Fig. 2G). The cytoplasmic area of Leydig cells is large and irregularly immunostained in a reticular pattern (Fig. 2I). Other cells within the interstitium of the testis are glucose-6-phosphatase immunonegative (Fig. 2G). In the developing seminiferous tubules, weak glucose-6-phosphatase immunoreactivity is variably present in some cells (Fig. 2G). Immunohistochemical staining in the absence of the glucose-6-phosphatase antibody resulted in no reactivity in Leydig cells or developing seminiferous tubules (Fig. 2H).

In the male, testosterone synthesis coincides with the appearance of Leydig cells and precedes the onset of virilization of the genital ducts (Saenger, 1984). In humans, Leydig cells appear at about 60 postovulatory days (Jirasek, 1977) and by 14 to 18 weeks gestation make up more than half the volume of the testis (Pelliniemi and Niemi, 1969). In the first trimester, Leydig cell function is regulated by human chorionic gonadotropin and in the second and third trimesters by the fetal pituitary. Soon after the eighteenth week, the number of Leydig cells decreases, and by 27 weeks the seminiferous tubules are separated by only a narrow interstitium containing few Leydig cells. Leydig cells are not visible histologically several weeks after birth, and they do not reappear until puberty (Jirasek, 1977). It is logical that, as in the adrenal, the presence of glucose-6-phosphatase is linked to steroidogenic activity.

Trachea and oesophagus

The presence of glucose-6-phosphatase activity in adult human intestine has been recognised for some time (Ockerman, 1964; Pears et al., 1992) (Table 1). The embryological origin of the epithelium of upper intestine, liver and associated structures pancreas, thyroid, trachea and lung is the same (O'Rahilly and Muller, 1987). Glucose-6-phosphatase activity has also been detected in adult rat pancreas (Waddell and Burchell, 1988) but its human development has not yet been studied. We also looked for glucose-6-phosphatase expression in other endodermal foregut derivatives and we found it in human trachea and oesophagus (Table 1).

There have also been several reports of glucose-6-phosphate hydrolysis in trachea (Rosen, 1970, 1972; Kanamura, 1975) using conventional histochemical methods based on the lead salt method (Chiquoine, 1953; Wachstein and Meisel, 1956). However, non specific hydrolysis of glucose-6-phosphate, other than that catalysed by glucose-6-phosphatase, remains a problem in histochemical assays of enzyme activity (Burchell and Waddell, 1991).

In the earliest embryos available (Stage 14, 32 postovulatory days), the structure of the oesophagus and the trachea are almost identical in appearance and both are glucose-6-phosphatase immunonegative. Mesenchymal development progresses rapidly during the remainder of the embryonic period (56 postovulatory days), with the formation of a wide submucosa and

external muscle layer around the oesophagus and with the condensation of mesenchymal cells, destined to become cartilage, antero-lateral to the tracheal epithelium (Fig. 3A). However, the epithelium of both trachea and oesophagus remain glucose-6-phosphatase immunonegative (Fig. 3A).

In fetal life at 9-10 weeks gestation, foci of glucose-6-phosphatase immunoreactivity are first present in the apices of luminal oesophageal epithelial cells and by 10 weeks of gestation nearly all apical cells are immunopositve (Fig. 3B). At 10-11 weeks gestation, occasional luminal oesophageal cells are ciliated but by 12 to 14 weeks gestation, the luminal cells are nearly all ciliated with the exception of the occasional non-ciliated cells and isolated or groups of mucus secretory cells. Glucose-6-phosphatase immunoreactivity is predominantly confined to ciliated cells, although non-ciliated luminal cells are also immunopositive and occasional basal cells show perinuclear staining (Fig. 3C). Mucus secretory cells are glucose-6-phosphatase immunonegative. Thereafter the numbers of luminal ciliated cells are in decline and are replaced by glucose-6-phosphatase immunonegative squamous cells (Fig. 3D), a process which results in an almost complete squamous epithelial lining by term.

The developing trachea follows a similar pattern to the oesophagus. Foci of glucose-6-phosphatase immunoreactivity are first present at 10-11 weeks gestation and by 11 weeks most luminal tracheal epithelial cells are glucose-6-phosphatase immunopositive. Occasional ciliated cells are apparent by 11-12 weeks gestation and rapidly increase in numbers over the next two weeks. Ciliated cells are strongly glucose-6-phosphatase immunoreactive, as are the smaller number of non-ciliated Clara cells, duct lining cells and the occasional basal cell (Fig. 3E). Mucus secretory cells are glucose-6-phosphatase immunonegative (Fig. 3E).

The expression of glucose-6-phosphatase in oesophagus is predominantly in ciliated cells, which are transiently present in fetal life, and specific activities measured at other stages in development presumably represents residual activity in the basal cell population (Pears et al., 1992). In contrast, as ciliated cells are normally one of the predominant epithelial cell types in the conductive airways, trachea retains glucose-6-phosphatase expression.

Brain

By far the most controversial and surprising location we have found glucose-6-phosphatase is in human brain. Glucose is the brain's most important substrate for energy metabolism and it has not previously been considered to be a gluconeogenic organ. Indeed the development of the use of the 2-deoxyglucose method in positron-emission tomography methods for the estimation of cerebral metabolic rates was based on the assumption that brain does not have glucose-6-phosphatase activity (e.g. Sacks et al., 1983; Fox, 1984; Nelson et al., 1986). Nevertheless, we found glucose-6-phosphatase immunopositivity in some but not all brain astrocytes (Bell et al., 1993). A number of histochemical studies have demonstrated that glucose-6-phosphate hydrolysis occurs in brain, neurones, oligodendroglia, astrocytes and many other cells but the interpretation is limited by the arguments about non-specific phosphatase described above (Stephens and Sandburn, 1976; Broadwell and Cataldo, 1984; Cataldo and Broadwell, 1986a,b; Balin and Broadwell, 1988; Pertsch et al., 1988).

Double immunofluorescence studies have shown that glial fibrillary acidic protein (GFAP) co-localises with glucose-6-phosphatase in some but not all astrocytes. In fetal brain, radial glia were consistently GFAP-positive but glucose-6-phosphatase negative. Many mature process bearing astrocytes in fetal and adult brain were positive for glucose-6-phosphatase, particularly processes ending on blood vessels. Glucose-6-phosphatase immunopositivity was also present in fetal ependyma and in the processes of small cells in the subependymal plate, choroid plexus epithelium and subpial glia limitants. In normal post-natal brains, many small astrocytes in white matter, but not in grey matter, were glucose-6-phosphatase positive both in cerebrum and cerebellum (Bell et al., 1993).

Limitations on the availability of sufficient amounts of fresh (i.e. not frozen) human brain for obvious reasons means that we have not attempted to assay glucose-6-phosphatase activity in human brain. However, we have confirmed that astrocytes isolated from rat brain contain specific microsomal glucose-6-phosphatase activity and glucose-6-phosphate transport (Forsyth et al., 1993). In addition, it has recently been shown that the human brain can export glucose under extreme and adverse conditions (Eyre et al., 1994). It is obvious that the role of glucose-6-phosphatase in astrocytes is not normally to produce glucose for the blood but it is possible that astrocytes have a function in local glucose production for nearby neurons.

Conclusions

Conventionally, the role of glucose-6-phosphatase was presumed to be production of glucose for the bloodstream in the gluconeogenic organs. However, from the studies described above, it is obvious that glucose-6-phosphatase has a wider distribution throughout the body and that this changes dramatically with development. Similarly, it is obvious in some cell types that the role of glucose-6-phosphatase is unlikely to be production of glucose for the blood, but it is not yet clear whether this is local glucose production or hydrolysis of other substrates. Further functional studies of the temporal and spatial expression of glucose-6-phosphatase in developing human tissues are now needed to resolve these questions.

Acknowledgments. The work carried out in Dundee was funded by a grant from the Scottish Home and Health Department (AB, RH), Scottish Hospital Endowment Research Trust (AB), National Kidney Research Fund (AB, RH), British Diabetic Association (AB) and the Wellcome Trust (RH), Sir Halley Stewart Trust (RH), Tenovus (Scotland) (AB,RH), Eli Lilly (AB), MRC (AB), Royal Society (AB), Anonymous Trust (AB, RH), Research Trust for Metabolic Diseases in Children (AB). AB was a Lister Institute Research Fellow. We thank Hazel Brewerton for her excellent technical assistance. The tissue collection for the studies in the author's laboratories was approved by the Pediatric Reproductive Medicine Ethics of Medical Research Sub-committee of Lothian Health Board, the Ethics Committee of Tayside Health Board and the Tayside Committee on Medical Research Ethics.

References

Allen J.M. (1961). The histochemistry of glucose-6-phosphatase in the epididymis of the mouse. J. Histochem. Cytochem. 9, 681-689.

Arion W.J., Ballas L.M., Lange A.J. and Wallin B.K. (1976). Microsomal membrane permeability and the hepatic glucose-6-phosphatase system. J. Biol. Chem. 251, 4901-4907.

Arion W.J., Lange A.J., Walls E.H. and Ballas L.M. (1980). Evidence for the participation of independent translocases for phosphate and glucose-6-phosphate in the microsomal glucose-6-phosphatase system. J. Biol. Chem. 255, 10396-10406.

Ashmore J. and Weber G. (1959). The role of hepatic glucose-6-phosphatase in the regulation of carbohydrate metabolism. Vitam. Horm. 17, 91-132.

Balin B.J. and Broadwell R.D. (1988). Transcytosis of protein through the mammalian cerebral epithelium and endothelium. 1. Choroid plexus and the blood-cerebrospinal fluid barrier. J. Neurocytol. 17, 809-826.

Barham S.B., Berlin J.D. and Brackeen R.B. (1976). The fine structural localization of testicular phosphatases in man: the control testis: Cell Tissue Res. 166, 497-510.

Baust V.P., Goslar H.G. and Tonutti E. (1967). Das verhalten einiger phosphatasen intact microsomes hoden von ratte. Maus und meerschwienchen nach oestrogenbehandlung. Acta Histochem. 26, 345-350.

Bell J.E., Hume R., Bussuttil A. and Burchell A. (1993). Immunocytochemical detection of microsomal glucose-6-phosphatase in human brain astrocytes. Neuropathol. Appl. Neurobiol. 19, 429-435.

Benkoel L., Gulian J.M., Payan M.J., Choux R., Brisse J. and Chamlian A. (1989). Cytochemical demonstration of glucose-6-phosphatase in human liver. Cell. Mol. Biol. 35, 563-571.

Berteloot A. and Hugan J.S. (1975). Effect of glutaraldehyde and lead on the activity of hepatic glucose-6-phosphatase. A biochemical and cytochemical study. Histochemistry 43, 197-214.

Bogitsh B.J. (1974). Cytochemical observations on the midpiece of the spermatozoa of the gastropod *Biomphalaria glabrata*. Trans. Am. Micros. Soc. 93, 211-219.

Brenner U., Hacker H.J. and Bannasch P. (1979). Electron microscopical demonstration of glucose-6-phosphatase in native cryostat sections fixed with glutaraldehyde through semipermeable membranes. Histochemistry 65, 41-47.

Broadwell R.D. and Cataldo E.E. (1984). The microsomal endoplasmic reticulum: its cytochemistry and contribution to the endomembrane system II. J. Comp. Neurol. 230, 231-248.

Burchell A. (1992). The molecular basis of the type 1 glycogen storage diseases. Bioessays 14, 395-400.

Burchell A. and Hume R. (1995). The human fetal testis endoplasmic reticulum glucose-6-phosphatase enzyme protein. (Submitted).

Burchell A. and Leakey J.E.A. (1988). Development of the rat hepatic microsomal glucose-6-phosphatase system and it's glucocorticoid inducibility. Biol. Neonate 54, 107-115.

Burchell A. and Waddell I.D. (1990). Genetic deficiencies of the hepatic microsomal glucose-6-phosphatase system. In: Genetics and human nutrition. Randle P.J., Bell J.I. and Scott J. (eds). John Libbey. London. pp 93-110.

Burchell A. and Waddell I.D. (1991). The molecular basis of the hepatic microsomal glucose-6-phosphatase system. Biochim. Biophys. Acta 1092, 129-137.

Burchell A. and Waddell I.D. (1993). The molecular basis of the genetic deficiencies of five of the components of the glucose-6-phosphatase system. Eur. J. Pedr. 152 (Suppl. 1), 518-521.

Burchell A., Waddell I.D., Countaway J.L., Arion W.J. and Hume R. (1988a). Identification of the human hepatic microsomal glucose-6-phosphatase enzyme. FEBS Lett. 242, 153-156.

Burchell A., Hume R. and Burchell B.B. (1988b). A new microtechnique for the analysis of the human hepatic microsomal glucose-6-phosphatase system. Clin. Chem. Acta 173, 183-192.

Burchell A., Gibb L., Waddell I.D., Giles M. and Hume R. (1990). The ontogeny of the human hepatic glucose-6-phosphatase proteins. Clin. Chem. 36, 1633-1637.

Burchell A., Lyall H., Busuttil A., Bell J. and Hume R. (1992). Glucose metabolism and hypoglycaemia in SIDS. In: Sudden Infant Death Syndrome. Busuttil A. and Burchell A. (eds). J. Clin. Pathol. 45 (Suppl.), 39-45.

Burchell A., Allan B.B. and Hume R. (1994). The endoplasmic reticulum glucose-6-phosphatase proteins. Mol. Membrane Biol. 11, 217-227.

Cataldo A.M. and Broadwell R.D. (1986a). Cytochemical identification of cerebral glycogen and glucose-6-phosphatase activity. J. Neurocytol. 15, 511-524.

Cataldo A.M. and Broadwell R.D. (1986b). Cytochemical identification of cerebral glycogen and glucose-6-phosphatase activity under normal and experimental conditions. J. Elect. Micr. Tech. 3, 413-437.

Chamlian A., Benkoel L., Bongrand P., Gulian J.M. and Brisse J. (1991). Quantitative histochemical study of glucose-6-phosphatase in periportal and perivenous human hepatocytes. Cell Mol. Biol. 37, 183-190.

Chen Y-T. (1991). Type I glycogen storage disease: kidney involvement, pathogenesis and its treatment. Pediatr. Nephrol. 5, 71-76.

Chen Y-T. and Burchell A. (1995). Glycogen storage diseases. In: The metabolic basis of inherited disease. Scriver C.R., Beaudet A.L., Sly W.S. and Valle D. (eds). 7th edn. McGraw-Hill. pp 935-965.

Chen Y-T., Coleman R.A., Sheinman J.I., Kolbede P.C. and Sidbury J.B. (1988). Renal disease in type I glycogen storage disease . N. Engl. J. Med. 318, 7-11.

Chiquoine A.D. (1953). The distribution of glucose-6-phosphatase in the liver and kidney of the mosue. J. Histochem. Cytochem. I, 429-439.

Colilla W., Jorgenson R.A. and Nordlie R.C. (1975). Mammalian carbamyl phosphate: glucose phosphotransferase and glucose-6-phosphate phosphohydrolase: extended tissue distribution. Biochim. Biophys. Acta 377, 117-125.

Cori G.T. and Cori C.F. (1952). Glucose-6-phosphatase of the liver in glycogen storage disease. J. Biol. Chem. 199, 661-667.

Essner E. (1973). Phosphatases. In: Electron microscopy of enzymes. Principals and methods. Hayat M.A. (ed). Vol. I. New Nork. Van Nostrand Reinold. pp 44.

Eyre J.A., Stuart A.G., Forsyth R.J., Heaviside D. and Bartlett K. (1994). Glucose export from the brain in man; evidence for a role for astrocytic glycogen as a resorvoir of glucose for neural metabolism. Brain Res. 635, 349-352.

Fantl P. and Rome N.M. (1945). Dephosphorylation in liver extracts. Aust. J. Exp. Biol. Med. Sci. 23, 21-27.

Forsyth R.J., Bartlett K., Burchell A., Scott H.M. and Eyre J. (1993). Astrocytic glucose-6-phosphatase and the permeability of brain microsomes to glucose-6-phosphate. Biochem. J. 294, 145-151.

Fox J.L. (1984). PET scan controversy aired. Science 224, 143-144.

Fulceri R., Romani A., Pompella A. and Benedetti A. (1990). glucose-6-phosphate stimulation of MgATP-dependent Ca^{2+} uptake by rat kidney microsomes. Biochim. Biophys Acta 1022, 129-133.

Fulceri R., Bellomo G., Gamberucci A., Scott H.M., Burchell A. and Benedetti A. (1992). Permeability of rat liver microsomal membrane to glucose-6-phosphate. Biochem. J. 286, 813-817.

Golbus M.S., Simpson T.J., Karesawa M., Appelne Z. and Alpers C.E. (1988). The prenatal determination of glucose-6-phosphatase activity by fetal liver biopsy. Prenatal Diag. 8, 401-404.

Hefferan P.M. and Howell R.R. (1977). Genetic evidence for the common identity of glucose-6-phosphatase, pyrophosphate-glucose phosphotransferase, carbamyl phosphate-glucose phosphotransferase and inorganic pyrophosphatase. Biochim. Biophys. Acta 496, 431-435.

Hermo L., Green H. and Clermont Y. (1991). Golgi apparatus of epithelial principle cells of the epididymal initial segment of the rat: structure, relationship with endoplasmic reticulum, and role in the formation of secretory vesicles. Anat. Rec. 229, 159-176.

Hers H.G. and De Duve C. (1950). Le système hexose-phosphatasique. II Répartition de l'activité glucose-6-phosphatasique dans les tissus. Bull. Sté. Chim. Biol. 32, 20-29.

Hilf R., Burnett F.F. and Borman A. (1962). The effect of ACTH upon adrenal glucose-6-phosphate metabolising enzymes. Biochem. Biophys. Res. Commun. 8, 191-194.

Hill A., Waddell I.D., Hopwood D. and Burchell A. (1989). The microsomal glucose-6-phosphatase system in human gallbladder. J. Pathol. 158, 53-56.

Hume R. and Burchell A. (1993). Abnormal expression of glucose-6-phosphatase in preterm infants. Arch. Dis. Child. 68, 202-204.

Hume R. and Burchell A. (1995). The glucose-6-phosphatase enzyme in developing human trachea and oesophagus. (Submitted).

Hume R., Lyall H. and Burchell A. (1992). Impairment of the activity of the microsomal glucose-6-phosphatase system in premature infants. Acta Paediatr. 81, 580-584.

Hume R., Bell J.E., Hallas A. and Burchell A. (1994). Immunohistochemical localisation of glucose-6-phosphatase in developing human kidney. Histochemistry 101, 413-417.

Hume R., Coughtrie M.W.H. and Burchell B. (1995a). Regional localisation of UDP-glucuronosyltransferase in kidney during human embryonic and fetal development. Arch. Toxicol. 69, 242-247.

Hume R., Voice M., Pazouki S., Giunti R., Benedetti A. and Burchell A. (1995b). The human adrenal microsomal glucose-6-phosphatase system. J. Clin. Endocrinol. Metabol. 80, 1960-1966.

Hume R., Pazouki S., Hallas A. and Burchell A. (1995c). The ontogeny of the glucose-6-phosphatase enzyme in human embryonic and fetal red blood cells. Early Hum. Dev. 42, 85-95.

Hume R., Burchell B., Allan B.B., Wolf C.R., Kelly R., Hallas A. and Burchell B. (1995d). The ontogeny of key endoplasmic reticulum proteins in human embryonic and fetal red blood cells (submitted).

Hume R., Brewerton H. and Burchell B. (1995e). The human embryonic-fetal kidney endoplasmic reticulum phosphate-pyrophosphate transport protein. (submitted).

Jirasek J.E. (1977). Morphogenesis of the genital system in the human. Birth Defects 13, 13.

Jonadet M., Turchini J.P. and Bastide P. (1968). Activité glucose-6-phosphatasique des glandes surrénales chez le Lapin (étude biochimique et histoenzymologique). Compt. Rend. Soc. Biol. 162, 1802-1805.

Jonges G.N., Van Noorden C.J.F. and Gossrau R. (1990). Quantitative histochemical analysis of glucose-6-phosphatase activity in rat liver using an optimised cerium-diaminobenzidine method. J. Histochem. Cytochem. 38, 1413-1419.

Kaliecharan D., Hulstaert C.E. and Hardonk M.J. (1985). Prevention of penetration hindrance in cerium-based glucose-6-phosphatase cytochemistry by freezing tissue in melting nitrogen. Histochemistry 82, 287-292.

Kanai K., Asada-Kubota M. and Kanamura S. (1981). Ultrastructural localization of glucose-6-phosphatase activity in the cells of the epididymis of the mouse. Experientia 37, 509-511.

Kanai K., Kanamura S., Watanabe J., Asada-Kubota M. and Yoshikawa M. (1983). Effect of castration and testosterone replacement on high glucose-6-phsophatase activity in principle cells of the mouse epidydimis. Anat. Rec. 207, 289-295.

Kanai K., Kanamura S. and Watanabe J. (1986). High and testosterone-dependent glucose-6-phosphatase activity in epithelium of mouse seminal vesicle. J. Histochem. cytochem. 34, 1207-1212.

Kanamura S. (1971). Fine structural demonstration of hepatic glucose-6-phosphatase activity after perfixation of fresh frozen sections in glutaraldehyde. J. Histochem. Cytochem. 19, 320-323.

Kanamura S. (1975). Ultrastructural localisation of glucose-6-phosphatase activity in the tracheal epithelium of the rat. J. Anat. 119, 499-504.

Maly I.P. and Sasse D. (1983). A technical note on the histochemical demonstration of glucose-6-phosphatase activity. Histochemistry 78, 409-411.

Marchesi V.T. and Palade G.E. (1967). The localization of Mg-Na-K-activated adenosine triphosphatase on red cell ghost membranes. J. Cell Biol. 35, 385-404.

Meusers P.J. (1966). Fermenttopochemische untersuchungen an der nebennierenrinde des meersschweinchens nach abschluss einer langfristigen cortisonvorbehandlung. Histochemie 7, 50-63.

Nelson T., Lucignani G., Goochee J., Crane A.M. and Sokoloff L. (1986). Invalidity of criticisms of the deoxymethod based on alleged glucose-6-phosphatase activity in brain. J. Neurochem. 46, 905-919.

Nordlie R.C. (1976). Glucose-6-phosphatase phosphotransferase. In: gluconeogenesis: its regulation in mammalian species. Hanson R.W. (ed). Wiley & Sons. pp 93-152.

Nordlie N.C. (1985). Fine tuning of blood glucose concentrations. TIBS 10, 70-78.

Nordlie R.C., Scott H.M., Waddell I.D, Hume R. and Burchell A. (1992). Analysis of human hepatic microsomal glucose-6-phosphatase in clinical conditions where the T2 pyrophosphate/phosphate transport protein is absent. Biochem. J. 281, 859-863.

O'Rahilly R. and Muller F. (1987). Developmental stages in human embryos. Publication 637. Carnegie Institution of Washington,

Washington DC.

Ockerman P.A. (1964). Glucose-6-phosphatase in human jejunal mucosa lack of activity in glycogenosis of Cori's type I. Clin. Chim. Acta 9, 151-156.

Oski F.A. (1993). The erythrocyte and its disorders. In: Hematology of infancy and childhood. 4th edition. Nathan D.G. and Oski F.A. (eds). W.B. Saunders. Philadelphia. pp 18-43.

Pears J., Jung R.T., Jankowski J., Waddell I.D. and Burchell A. (1992). Glucose-6-phosphatase in normal adult human intestinal mucosa. Clin. Sci. 83, 683-687.

Pelliniemi L.J. and Niemi M. (1969). Fine structure of the human foetal testis. 1. the interstitial tissue. Z. Zellforsch. Mikrosk. Anat. 99, 507.

Pertsch M., Duncan G.E., Stumpf W.E. and Pilgrim C. (1988). A histochemical study of the regional distribution in the rat brain of enzymatic hydrolyzing glucose and 2 deoxyglucose-6-phosphatase. Histochemistry 88, 257-262.

Restaino I., Kaplan B.S., Stanley C. and Baker L. (1993). Nephrolithiasis, hipocitraturia, and a distal renal tubular acidification defect in type I glycogen storage disease. J. Pediatr. 122, 392-396.

Rosen S.I. (1970). A histochemical survey of the glucose-6-phosphatase hydrolysing enzyme activity in the RES: a light microscopic study. Acta Histochem. 38, 199-217.

Rosen S.I. (1972). Preliminary observations on a glucose-6-phosphate-hydrolysing enzyme in secretory cells: a light microscopic study. Histochem. J. 4, 127-134.

Sacks W., Sacks S. and Fleischer A. (1983). A comparison of the cerebral uptake and metabolism of labelled glucose and deoxy glucose in vivo in rats. Neurochem. Res. 8, 661-685.

Saenger P. (1984). Abnormal sex differentiation. J. Pediatr. 104, 1-17.

Simpson T.J., Karesawa M., Hogge W.A., Holzgerne W. and Golbus M.S. (1987). Ontogeny of fetal liver glucose-6-phosphatase activity. Prenatal Diag. 7, 639-651.

Stephens H.R. and Sandburn E.M. (1976). Cytochemical localisation of glucose-6-phosphatase activity in the central nervous system of the rat. Brain Res. 113, 127-146.

Thiery G., Bernier J. and Bergeron M. (1990). A new method based on cobalt for histochemical and cytochemical demonstration of glucose-6-phosphatase activity. J. Histochem. Cytochem. 38, 1503-1509.

Thomas D.B. and Yoffey J.M. (1964). Human foetal haematopoiesis. II. Hepatic haematopoiesis in the human foetus. Brit. J. Haematol. 10, 193-198.

Thorne-Tjomsland G., Clermont Y. and Tang X. (1991). Glucose-6-phosphatase activity of endoplasmic reticulum and Golgi apparatus in spermatocytes and spermatids of the rat: an electron microscopic cytochemical study. Biol. Cell 71, 33-41.

Vilar O., Perex del Cerro M.I. and Mancini R.E. (1962). The sertoli cell as a «bridge cell» between the basal membrane and the germinal cells. Exp. Cell Res. 27, 158-161.

Wachstein M. and Meisel E. (1956). On the histochemical demonstration of glucose-6-phosphatase. J. Histochem. Cytochem. 4, 592.

Waddell I.D. and Burchell A. (1988). The microsomal glucose-6-phosphatase enzyme of pancreatic islets. Biochem. J. 255, 471-476.

Waddell I.D., Hume R. and Burchell A. (1989). A direct method for the diagnosis of human hepatic type Ib and type Ic glycogen-storage disease. Clin. Sci. 76, 573-579.

Waddell I.D., Scott H., Grant A. and Burchell A. (1991). Identification and characterisation of a hepatic microsomal glucose transport protein. Glucose-6-phosphate T3? Biochem. J. 275, 363-367.

Waddell I.D., Zomerschoe A.G., Voice M.W. and Burchell A. (1992). Cloning and expression of a hepatic microsomal glucose transport protein: comparison to GLUT 2. Biochem. J. 285, 527-532.

Weber G. and Cantero A. (1955a). Glucose-6-phosphatase activity in regenerating embyronic, and newborn rat liver. Cancer Res. 15, 679-684.

Weber G. and Cantero A. (1955b). Glucose-6-phosphatase activity in normal precancerous, and neoplastic tissues. Cancer Res 15, 105-108.

Wegmann R. and Khosrovchahi H. (1964). Variations d'activités histoenzymologiques au cours de la stéroïogénèse surrénalienne chez la ratte. Ann. Histochim. 9, 57-80.

Yokoyama M. and Chang J.P. (1977). Cytochemical study of glucose-6-phosphatase in chinese hamster testis. Biol. Reprod. 17, 265-268.