CHAPTER II

LITERATURE REVIEW

Cadmium

History of cadmium

Cadmium (Cd) was discovered in as an element in 1817 in Germany. The first effect of Cd on human health was the damage to the lungs in Cd-exposed workers, published in 1938. In later, Cd exposure effected to pathological bone fractures and severe pain (named Itai-Itai disease) occurred after World War II, in Toyama, Japan (Nordberg, et al., 2004). At the present, Cd has been still an important in industrial metal. It was extracted during the production of several metals such as zinc (Zn) and spread in environmental and produces toxic effects on humans (ATSDR, 1998).

Physical and chemical properties of Cadmium

Cd, atomic number 48 and relative atomic mass 112.41 $g{\cdot}mol^{-1}$, is a toxic metal that belongs, together with zinc and mercury, to group IIb in the Periodic Table. Naturally occurring isotopes are 106 (1.22%), 108 (0.88%), 110 (12.39%), 111 (12.75%), 112 (24.07%), 113 (12.26%), 114 (28.86%), and 116 (7.50%). Melting point and boiling point are 321 °C and 767 °C respectively (WHO, 1992; ATSDR, 2008; ATSDR, 1998).

Pure Cd is a soft and silver-white metal. Cd does not usually present in the environment in its pure form, but as a mineral compound with other elements such as oxygen (cadmium oxide, CdO), chlorine (cadmium chloride, $CdCl_2$), or sulfur (cadmium sulfate, $CdSO_4$). $CdCl_2$ and $CdSO_4$ are soluble in water. It occurs in nature at low concentrations in zinc, lead, and copper ores. Most Cd used in the United States is extracted as a byproduct during the production of other metals such as zinc, lead, or copper. Cd is recovered from used batteries. Cd is used for the following: batteries (83%), pigments (8%), coatings and platings (7%), stabilizers for plastics (1.2%), nonferrous alloys, photovoltaic devices, and other uses (0.8%) (WHO, 1992; ATSDR, 2008; ATSDR, 1998).

Major sources of Cd

Cd is a contaminant that affects many different areas of the environment. It contaminates in soil and is absorbed and gradually accumulated by plants (Nordberg, et al., 2007; Simmons, et al., 2005). The discovery of Cd contamination to rice and soil in Thailand began in 1998 (IWMN, 2003). Recently, Cd has been discovered in environment in Mae Sot District, Tak Province, and Northwestern Thailand. The results indicate that the contamination is associated with suspended sediment transported to fields via the irrigation supply. Over 90% of the rice grain samples collected from contaminate areas contained Cd at concentrations exceeding 0.2 mg Cd kg^{-1}.In addition, estimated Weekly Intake (WI) values ranged from 20 to 82 g Cd per kg Body (Simmons, et al., 2005; Swaddiwudhipong, et al.,2007).

The uptake of Cd in human body occurs via food intake and smoking (Satarug and Moore, 2004; Satarug, et al., 2004). The Cd has a long half-life in the human body is approximately 10–30 years. A small part of Cd that enters body leaves slowly in urine and feces (ATSDR, 2008). The major source of Cd contamination is food which is able to expose in the general population. From the available data showed that the Cd intake from the diet is most commonly 10-35 μg per day. The elevated levels of Cd in water are a contributor to Cd intake for most individuals and can be as large as the dietary intake. Not only by ingestion, but most of people can also intake Cd by the inhalation, for example smoking 20 cigarettes per day may give a further 1-4 μg of Cd per day (WHO, 1989).

In 1989, the Food and Agriculture Organization/World Health Organization (FAO/WHO) Joint Expert Committee on Food Additives (JECFA) set the provisional tolerable weekly intake (PTWI) for Cd at 7μg/kg body weight/week, corresponding to 1 μg/kg body weight/day, or 70 μg/day (WHO, 1989, WHO, 1999).World Health Organization (WHO) set standard urinary Cd for environmental exposure at 2 μg/g creatinine, occupational exposure at 5 μg/g creatinine and possible renal damage caused by cadmium at > 10 μg/g creatinine (Padungtod, 2007).

Cd adsorptions in the body

There are three possible ways of Cd absorptions: Gastrointestinal, pulmonary and dermal (Satarug, et al., 2004; Godt, et al., 2006).

1. **Gastrointestinal tract absorption**

The dietary Cd absorption rate in humans has been estimated at 5% (WHO, 1989) by gastrointestinal tract (GI), specifically the duodenum. This absorption rate depends on the exact dose and nutritional composition. However, several factors can increase Cd absorption such as low intakes of vitamin D, calcium, and trace elements like iron zinc and copper. The DMT-1, has been shown to be involved in Cd absorption in intestinal tract. The body iron depletion and increasing Cd uptake from GI tract due to upregulated DMT-1 protein in intestine and circulated to body tissues (Park, et al., 2002; Kim, et al., 2007).

2. **Pulmonary absorption**

Cd is a one of tobacco component. CdO is generated during the burning of cigarettes. The human lung can absorb 40–60% of the Cd in tobacco smoke (Elinder, et al., 1976). Cd is absorbed into blood circulation usually in form of Cd-cysteine complexes (Zalups, et al., 2003).

Handling of cadmium in the body

After Cd uptakes into the body and be transported by bound to proteins, such as albumin and metallothionein in blood. They reach to liver where further formed Cd-Metallothionein (Cd-MT) complexes. These Cd-MT complexes are accumulated in hepatocytes and induced hepatocyte necrosis and apoptosis. Cd is excreted to biliary tract in form of Cd-Glutathione conjugates and degraded to Cd-cysteine complexes which re-enters the small intestines (Zalups, et al., 2003). The long term of Cd accumulation effects on kidney, resulting in tubular cell necrosis. Excretion of Cd takes place via feces and urine, principal route of excretion is via the urine. The average daily excretions of Cd for humans being are about 2 to 3 µg (ATSDR, 1989). The handling of Cd in human body is shown in Figure 1 (Godt, et al., 2006).

Health effects of cadmium

1. Acute intoxication

The acute toxicity of Cd results in lung edema and destruction of mucous membranes as part of Cd-induced pneumonitis (Seidal, K. et al., 1993). ATSDR in 1989 reported lower exposure of Cd causes of gastrointestinal irritation, vomiting, abdominal pain, and diarrhea. The gastrointestinal effects from acute Cd ingestion are at doses above about 0.07 mg/kg. In humans, the symptoms of Cd overdoses are nausea, vomiting, diarrhea and abdominal pain (Nordberg, et al., 2004).

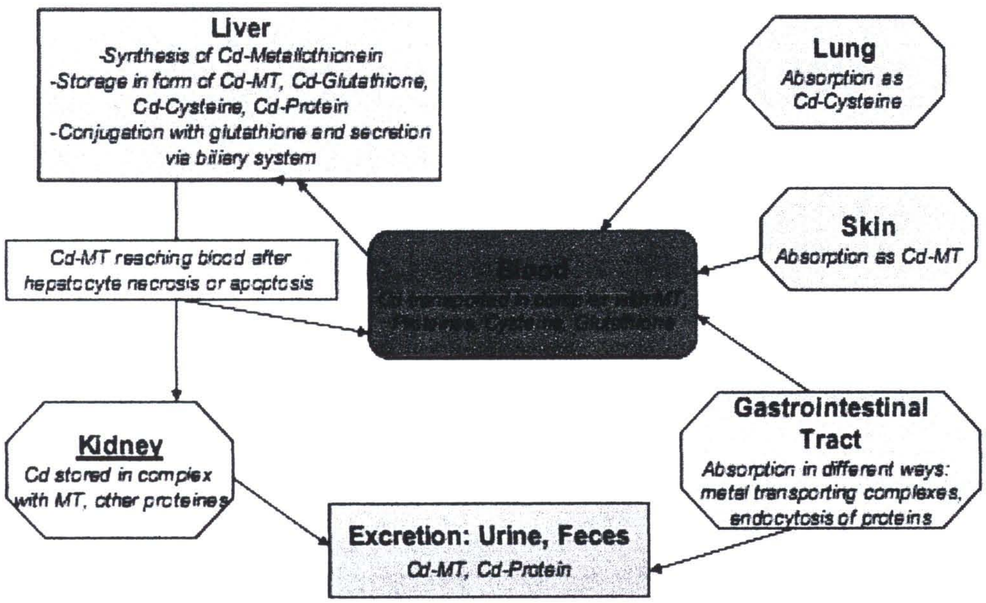


Figure 1 Handling of cadmium in human body

Source: Godt, et al., 2006

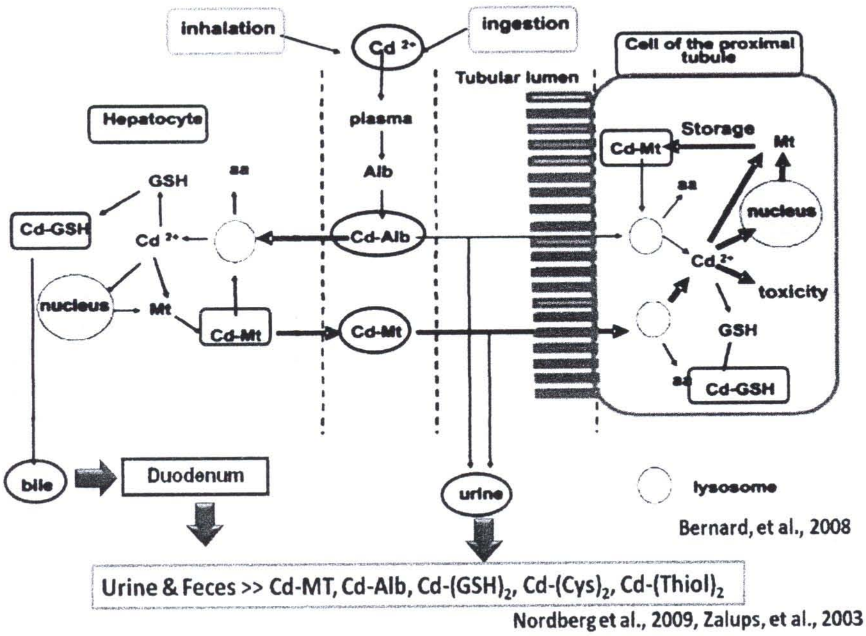


Figure 2 The cadmium mechanism in proximal tubular cells and hepatocyte. Alb, albumin; Mt, metallothionein; GSH, glutathione; aa, amino acid.

Source: Modified from Bernard, et al., 2008; Norberg, et al., 2009; Zalups, et al., 2008

2. Renal effect

The glomerular filtration pathway is at the origin of the selective accumulation of Cd. Because small molecule size of Cd-Mt is easily filtered through glomerulus and absorbed to renal tubular tubule (Nordberg, et al., 1978). Renal toxicity (tubular proteinosis) may also effect from inhalation exposure to Cd (Goyer, et al., 1991).The acute Cd exposure in rat was studied in 2007, has shown that $CdCl_2$ administration increased tissue damage in kidney, proximal and distal tubules degenerated as time passed. Increased apoptosis was seen in the proximal tubules epithelium, especially on day 7. The effects of exogenous metallothionein (MT) were studied with Cd exposure in rat. They found that the structural changing of kidney that increased depending on the day of Cd administration decreased after exogenous MT administration. These evidences were interpreted that most renal Cd is bound to MT, is a Cd-inducible protein that protects the cell by tightly binding the toxic Cd^{2+} ion (Kukner, et al., 2007, Bernard et al., 2008).The result of kidney damage leads to Cd releasing in the urine. The previous study was suggested that the impairment of glomerulus linked with the urinary Cd 0.8 μg/g creatinine (Akesson, et al., 2005).

3. Hepatic effects

Previous study about the exposure of Cd by drinking in rat indicated that Cd significantly accumulated in liver and leading to histological changes including light cytoplasm, enlarged cell size, condensed nuclear chromatin, sinusoidal widening and accumulation of mononuclear cells in vicinity of sinusoids and hepatocytes necrosis (Jihen, et al., 2008). Then showed data on renal dysfunction biomarkers, they found that in the high Cd burden group showed higher rates of urinary of excretion of β2-microglobulin (β_2-MG) and N-acetyl-β-D glucosaminidase (NAG) than did the average Cd burden group. So, they suggested that Cd exposure is associated with increases in prevalence of high blood pressure and signs of renal tubular damage (Satarug, et al., 2005).

4. Musculoskeletal effects

The effects of Cd on the bones happened with the spread of the Itai-Itai disease in the Cd contaminated area of Toyama, Japan, after World War II (Nicaud, et al., 1942). The symptoms of Itai-Itai disease are severe osteomalacia follow with multiple bone fractures, renal dysfunction, pain in the back and in the extremities, difficulties in walking and pain on bone pressure. Hence, the name Itai-Itai is also known as Ouch-Ouch in Japanese (Friberg, et al., 1950). Osteoporosis and osteomalacia in Cd workers have been reported from England, Poland, Russia (Nicaud, et al., 1942; Nordberg, et al., 2007).

5. Hematological effects

Both Hemolytic anemia (PHA) and Fe-deficiency anemia (FeDA) are abnormal of iron metabolism. After Cd administration, DMT-1 expression, hepatic and renal Cd concentrations were significantly increased in both FeDA and PHA. This suggests that anemia may be a risk factor for Cd accumulation (Kyong-Son, et al., 2008). Okubo who studied of Xenopus leavis oocytes, it has been shown that DMT-1 transports both Fe and Cd. In Fe-deficient WT mice, DMT-1 mRNA was increased and iron deficiency resulted in upregulation of Cd intake in the intestine (Suzuki, et al., 2008).

6. Cardiovascular effects

Low-dose Cd exposure associated with the increase of risk factor of peripheral arterial disease (PAD). The subjects with PAD had higher urinary Cd than the subject without PAD (Navas, et al., 2004; Navas, et al., 2005). The subject who was highly urinary Cd is found that high risk for myocardial infarction (Everett, et al, 2008). The relationship between blood pressure and kidney damage caused by environmental exposure to the pollutants Cd and Pb was analysis by determine systolic/diastolic pressure together with Cd and Pb burden. The blood pressure status according to Wolrd Health Organization were normal systolic/diastolic values = normal systolic/diastolic values = 130/70 or less; mild hypertension systolic/diastolic values = $\geq$140/$\geq$80; obvious hypertension systolic/diastolic values = $\geq$150/$\geq$90. They found statistically significant differences in predominance rate of high blood pressure of 2%, 7.7% and 19%, respectively in the subjects with in low, average and high Cd burden ($p=0.01$)

7. Metabolic Effects.

Human male who had ingested 25 mg/kg Cd as cadmium iodide (CdI) were reported that they had hyperthermia and metabolic acidosis (Wisniewska, et al., 1971).

8. Neurological effects

The Cd implicated in neurological disorder. Central nervous system (CNS) is sensitive to disturbances of trace element concentration which necessary for brain development. Cd disturbs the metabolism of Cu and Zn (Me′dez-Armenta, et al., 2007). The synthesis of DMT-1 protein was induced by Pb and Cd exposure in rat CNS, indicated that DMT-1 has association for transport toxic metal in rat brain developing (Chengwu, et al., 2009). Pb and Cd can cause the CNS damaging such as intracellular reactive oxygen species (ROS), lipid peroxidation (Valverde, et al., 2001).

9. Reproductive effects

Alsberg and Schwartze in 1919 had shown the effects of Cd on reproductive tissues. The macroscopic and microscopic changes were found in the testes of several species of animals after Cd-exposure. Parizek and Zahor in 1956 also reported that testicular necrosis occurrence is also found. The ovarian changes are observed when moderate Cd doses were injected in rats (Kar, et al., 1959).

10. Developmental effects

Progesterone is known as the necessary hormone for pregnancy progression. It had been documented that the blood progesterone levels appeared to decrease in smokers and subject who expose to air pollution (Sorkun, et al., 2007; Piasek, et al., 2001). Whereas the mean count of syncytial knots, thickening of vasculo-syncytial membrane and MT staining were highly found in these subjects (Sorkun, et al., 2007). Osman and coworkers suggested that the reducing of Cd transferred from the mother to the fetus involved in the increasing of MT expression in placenta (Osman, et al., 2000). Necrosis and increased syncytial knots are hallmarks of hypoxia or degenerative changing due to ischemic conditions in placenta (Vander, et al., 1982). Previous study had reported that environmental exposure to Cd induced maternal and cord blood Cd levels, additional low birth weight (< 2,500g) (Tian, et al., 2009). The iron level reduced in placentas of mothers delivering low birth weight babies while Cd, lead and arsenic concentrations increased in placenta (Llanos et al., 2009). Toxic metals accumulation in placental tissue may outcome in abnormal placental function, leading to impaired nutrient transport and fetal growth restriction (Norberg, et al., 1998; Zadorozhnaja, et al., 2000). Cd can induce oxidative stress in pregnant. Placental is one of source for reactive oxygen species (ROS) that produce endothelial cell membrane damage like several pathological healths in pregnancy such as pre-eclampsia, pregnancy-induced diabetes and hypertension

11. Carcinogenity

Cd can cause several types of cancer such as pulmonary cancer (Waalkes, et al., 2003), prostate cancer in Wistar rats (Waalkes, et al., 1988), testicular necrosis, testicular interstitial tumor (Sahmoun, et al., 2005), renal cell cancer (Pesch, et al., 2000) and breast cancer (Band, et al., 2002).

Cd transporters in enterocyte

To date, transporters for iron (Fe), calcium (Ca), zinc (Zn), manganese (Mn), and magnesium (Mg) have been proposed to be involved in Cd uptake in mammalian cells (Himeno, et al., 2009). Since amino acid transporters have been implicated in the luminal absorptive transport of mercuric conjugates of cysteine (Cys) in the renal proximal tubule, and since some of the same transport systems are also present in enterocytes, it is reasonable to postulate that certain amino acid and oligopeptide transport systems may be involved in the absorptive transport of Cys-containing oligopeptide S-conjugates and/or Cys-S-conjugates of Cd along the small intestine. DMT-1 likely plays a key role in transporting Cd into enterocytes. This transporter, which is expressed in the luminal plasma membrane, is a proton-coupled transporter that appears to be involved in the intestinal absorption of non-heme ferrous (2+) iron and Cd (Zalups and Ahmad 2003). Elisma and Jumarie (2001) have also demonstrated that Zn and Cd compete for a luminal membrane transporter in enterocytes that is not only DMT-1. Zn transporter, especially Zrt-, Irt-related protein such as ZIP8, ZIP14 and ZnT1 which was reported that candidate for Cd transporter (Tallkvist, et al., 2001). The data indicate that Cd may also enter into enterocytes through one of the transport systems involved in the luminal uptake of Zn, perhaps at hZTL1 which is a human, zinc-regulated, transporter of zinc expressed in the luminal membrane of enterocytes (Cragg, et al., 2001). ZIP14, and CaT, a Ca transporter-1 specifically expressed in the intestine is high in the intestine, it seems likely that dietary intake of Fe, Ca, Zn, and Mn may influence the intestinal absorption of Cd. Moreover, the role of ZIP8, ZIP14, DMT1, and Ca channels in the liver, kidney, testis, and other organs may depend both on the expression levels of each transporter among tissues and on the carriers of each metal in the blood stream (Himeno, et al., 2009). It seems possible that Cd ions may enter into enterocytes through Ca channels (Zalups and Ahmad 2003).

On the other hand, the zinc transporter ZnT1 had been identified in the basolateral membrane of enterocytes (Palmiter and Findley, 1995). In addition, involvement of ZnT1 in Cd efflux, lowering of ZnT1 expression in cortical neurons caused an enhancement in Cd uptake with neuronal cell death (Ohana, et al., 2006). Immunohistochemical localization experiments indicate the appearance of MTP1 in the basolateral membrane and cytoplasm of enterocytes. MTP-1 or ferroportin-1, ZnT1, CaATPase is suggested that transport Cd efflux from enterocyte (Himeno, et al., 2009 and Zalups and Ahmad 2003). Possible pathways of Cd transport in enterocyte as shown in Figure 3.

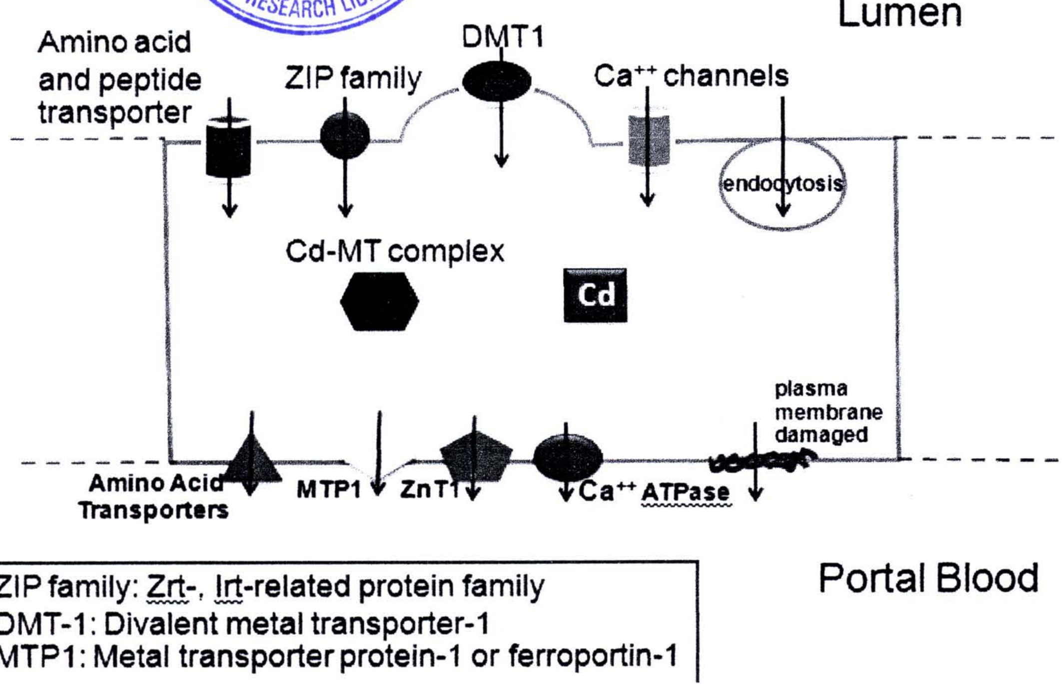


Figure 3 Possible pathways of Cd transport in enterocyte

Source: modified from Zalups and Ahmad, 2003

Placenta

General views of placenta

The relationship between the embryo and mother is one of the most characteristic features of human embryonic development. To survival and growth development of embryo, the embryo must maintain an essential communication with body of the mother for achieving oxygen, nutrient and expelling wastes. The placenta is a temporary materno-fetal organ formed during pregnancy from elements of the membranes which surround the developing fetus (uterine endometrium) and provides for physiological exchange between the fetal and maternal circulations. The placenta performs gaseous exchange, excretion, maintenance of homeostasis, hormone secretion, haemopoiesis and hepatic metabolic functions until the fetal organs become mature (Young, 2000; Donnelly and Campling, 2008; Carlson, 1999; Gude, et al., 2004)

Placental formation during fertilization and cleavage

Human fertilization is the union of a human egg and sperm occurs in the ampulla of the Fallopian tube within about 12 hours after ovulation. It is also the initiation of prenatal development (see Figure 4). The zygote suffers its first mitotic cell division within 24 hours and continuous to 4-5 days until there are some 20-50 cells called blastomeres. When the embryo consists of approximately 16 cells, it is called a morula (for its likeness to a mulberry) and enclosed by thinning zona pellucida. By now the morula has reached the uterus and begins to absorb uterine fluid forming a central cavity, called cavitation, and the space is known as the blastocole (Donnelly and Campling, 2008). The whole embryo is called blastocyst, consists of a peripheral layer of blastomeres forming the trophoblast which surrounds a small inner group of cells known as the inner cell mass. The trophoblast forms only outer layer of the placenta while the inner cell mass develops to the body of the embryo (Young, 2000; Donnelly and Campling, 2008; Carlson, 1999; Gude, et al., 2004).

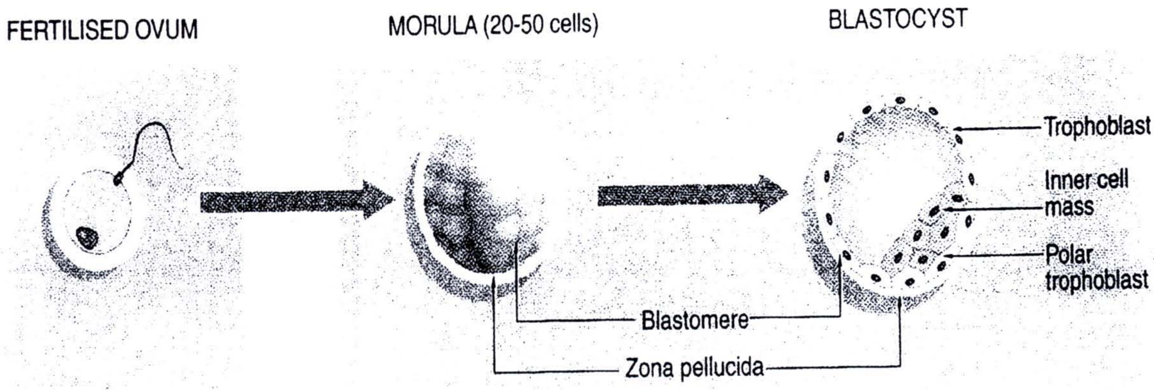


Figure 4 Fertilization and cleavage of the embryo

Source: Young and Heath, 2000

Placental formation during implantation

After fertilization as 6-8 days, the blastocyst remains in the uterine cavity and implants into midportion of posterior wall of the uterus. Destruction of the zona pellucida causes of the endometrium attach with the trophoblast which undergoes rapid growth and differentiation (Young, 2000; Donnelly and Campling, 2008; Carlson, 1999; Gude, et al., 2004).

During early human pregnancy, the trophoblast cells gives rise to two layers, an inner cytotrophoblast layer (mononuclear cells) which proliferates and fuses to form outer syncytiotrophoblast layer (multinucleate cells). The syncytiotrophoblast quickly expands into the endometrium (finger-like projections) and develops network of spaces called lacunae within the syncytiotrophoblast. Maternal blood begins to fill the lacunae. Progressively the trophoblast enfolds maternal capillaries and lacunar network, forming an arterial supply and venous drainage system (Young, 2000; Donnelly and Campling, 2008; Carlson, 1999; Gude, et al., 2004).

The syncytiotropoblast secretes a difference of hormones containing human chorionic gonadotrophin (HCG), human placental lactogen (HPL), estrogen and progesterone which are needful to maintain the endometrial tissues (see Figure 5).

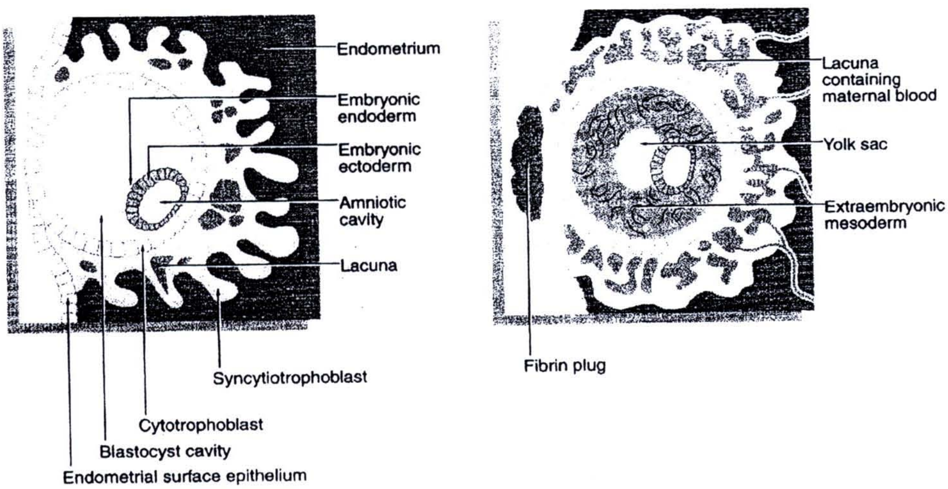


Figure 5 Implantation of the embryo

Source: Young and Heath, 2000

The trophoblastic tissue in previllous embryo period have no consonant morphological feature, subsequent, cytotrophoblastic cells invade to trabeculae between the lacunae called a primary chorionic villi. Extraembryonic mesoderm spreads to the primary villi which mesenchymal core appears, it is called a secondary chorionic villi. Therefore secondary chorionic villi including messenchymal core which surrounding cytotrophoblast layers and outside of that is syncytiotrophoblast. When blood vessel penetrates into mesenchymal cell of secondary chorionic, it is become known as tertiary villi. This occurs about the end of third week of pregnancy (Young, 2000; Donnelly and Campling, 2008; Carlson, 1999; Gude, et al., 2004).

The cytotrophoblastic cells expand distally and proliferates the syncytiotrophoblast layer, until abutting onto maternal decidual cells and spreads over them to form cytotrophoblastic shell. Most villi are free in the intervillous space and are bathed in blood from the maternal vessels. Anchoring villi contact the decidua basalis (Young, 2000; Donnelly and Campling, 2008; Carlson, 1999; Gude, et al., 2004) (See Figure 6).

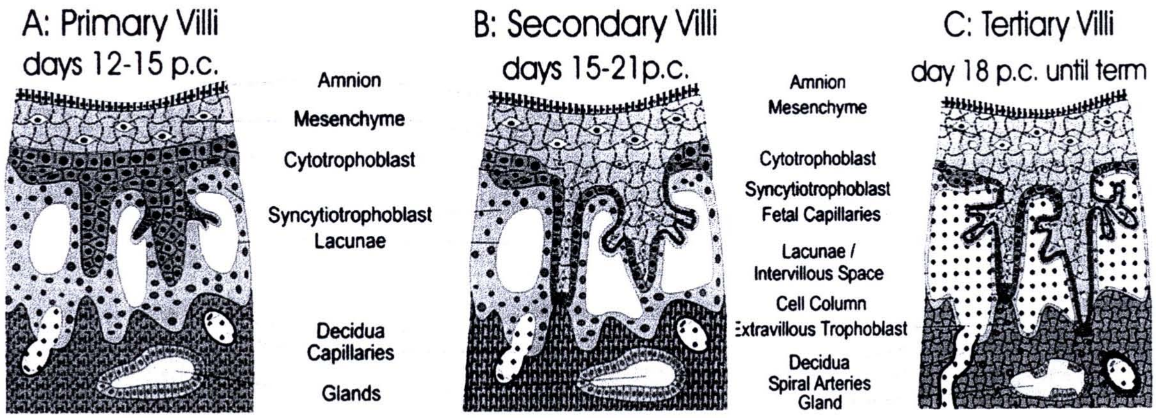


Figure 6 Stages of Chorionic villi. (A) Primary Chorionic villus (B) Secondary Chorionic villus and (C) Tertiary chorionic vilus.

Source: Chaddha, et al., 2004

Decidual formation and early placental development

The change of cellular and vascular in endometrium as the blastocyst implantation is referred to as decidual reaction. The decidual cell function is unclear but it is suggested to have main role in facilitating implantation and trophoblast migration. After implantation, the stromal cells of endometrial stratum grow and proliferate, these cells are known as decidual cells. The deciduas that below the development of embryo are known as the decidual basalis, it forms to the future placenta with the trophoblast cells. Deciduas cupsularis is decidual tissue that covers the embryo and its chorionic vesicle that surrounds the embryo, amnion, yolk sac and body stalk and the decidual lining on the side of the uterus is called the deciduas parietalis (Young and Heath, 2000). The chorion in contact with the deciduas basalis is known as the chorion frondosum. The superficial chorion in contact with the deciduas capsularis which final becomes the smooth is the chorion leave. Progressively, the chorion frondosum and deciduas basalis develop into the flattened placenta and the vessels connecting the chorion to the embryonic circulation become the umbilical cord (Young and Heath, 2000) (see Figure 7).

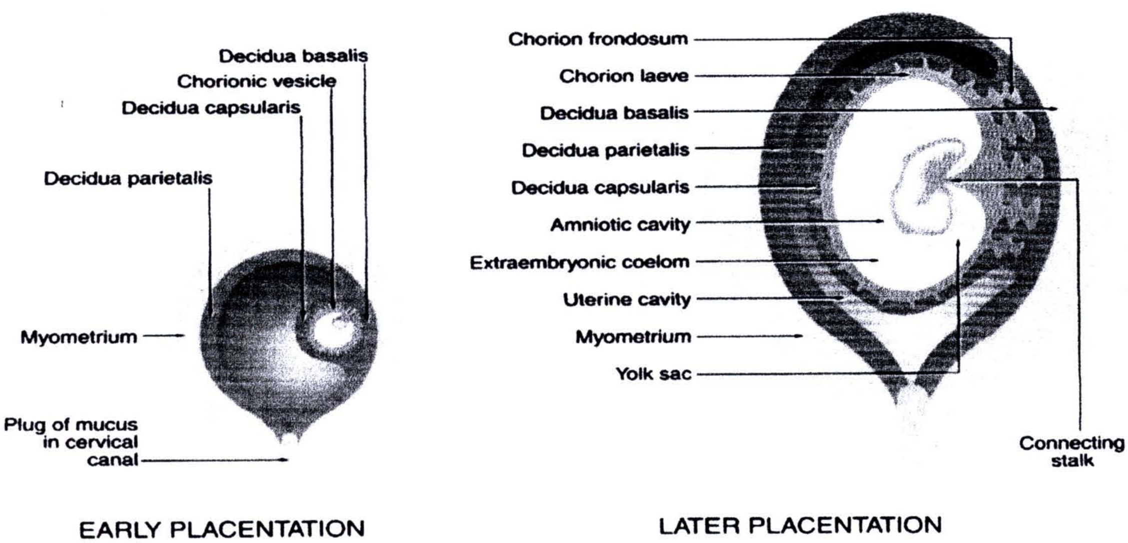


Figure 7 Decidua formation and early placental development

Source: Young and Heath, 2000

The structure of mature placenta

At term, the mature placenta is usually a disc shaped structure, as different from other mammalian species. The placenta is measuring approximately 20 cm. in diameter, 2.5 cm. in thickness and 500 grams in weight. It has a dark reddish-blue or maroon color. It connects to the fetus by an umbilical cord of approximately 55–60 cm. in length which contains two arteries and one vein. The utero-placental unit consists of both the fetal tissue and maternal tissue. The fetal portion of the placenta is called the chorionic plate. This region have fetal chorionic blood vessel, it branching radials from the umbilical vessel. The maternal portion of the placenta is decidua basalis and called the basal plate which villous tree structures are grouped into lobules by placental septum know as cotyledons, have estimably 20 cotyledon per placenta. In between these two regions is the intervillous space, which contains the villous structures containing fetal blood and filled maternal blood. Whole of the placenta are covered by the fetal membrane (fetal-facing amnion and maternal-facing chorion) (Young and Heath, 2000; Donnelly and Campling, 2008; Carlson, et al., 1999; Gude, et al., 2004) (See Figure 8).

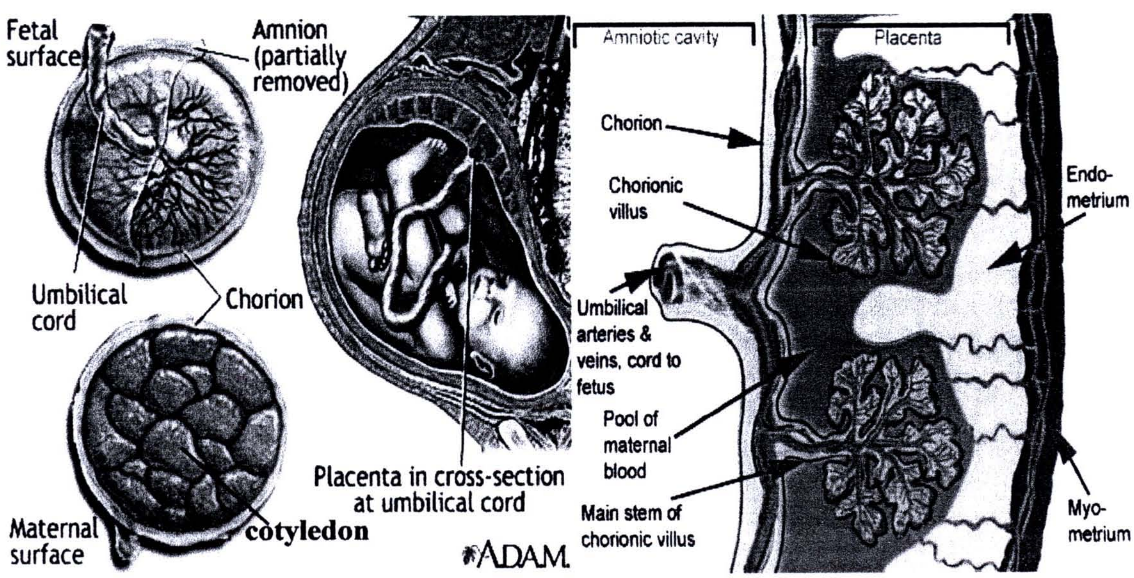


Figure 8 Placental feature and placental structure

Source: http://www.nlm.nih.gov/medlineplus/ency/images/ency/fullsize/17010.jpg and www.medicine.mcgill.ca/physio/vl...enta.jpg

Materno-fetal circulation

Spiral arteries in the deciduas are remodeled so that they become less coiled and increased their diameter. After remodeling, their resistances are decreased whereas the maternal blood flow to the placenta is increased. The high blood pressure of spiral arteries enters to the intervillus space and immerses the chorionic villi. As the pressure decreases, the deoxygenated blood drains back through the endometrial veins. Deoxygenate fetal blood passes through two umbilical arteries and branched chorionic artery to extensive arterio-capillary venous system. Oxygenate fetal blood in capillary return to the fetus via chorionic vein and the single umbilical vein (Young and Heath, 2000; Donnelly and Campling, 2008; Carlson, et al., 1999; Gude, et al., 2004) (See Figure 9).

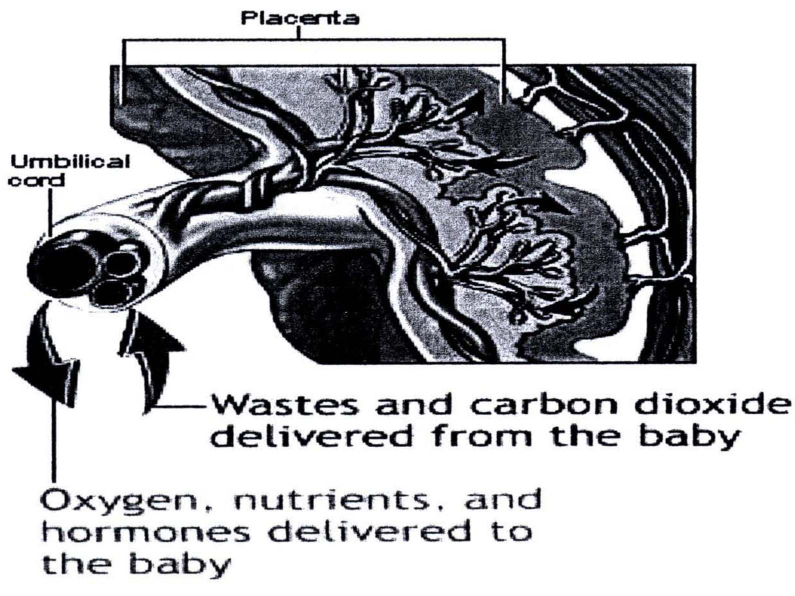


Figure 9 Blood circulation between mother and fetus

Source: http://www.walgreens.com/marketing/library/graphics/images/en/19505.jpg

The placental membrane

Definition of the placental membrane is semipermeable layer of tissue separating maternal blood from fetal blood in the placenta. It sometime is called the placental barrier. It consists of four cell layers in intervillous space. There are a layer of syncytiotrophoblast, a layer of cytotrophoblast, connective tissue and the endometrium of fetal capillary. The chorionic villus becomes at full term approximately 20 weeks. Placental barrier consists of three layers; syncytiotrophoblast, connective tissue (Mesenchymal tissue) of villus and endothelium of fetal capillaries. However, the cytotrophoblast layer becomes reduced and disappears (Young and Heath, 2000; Donnelly and Campling, 2008; Carlson, et al., 1999; Gude, et al., 2004) (See Figure 10). In some areas the placental membrane becomes markedly thinned and attenuated. At these sites, the syncytiotrophoblast comes in direct contact with the endothelium of the fetal capillaries (Gude, N.M., et al. 2004). Therefore, in this area, the maternal blood and fetal blood turn into extremely close proximity (approximately 2—4 μm) as shown in Figure 10.

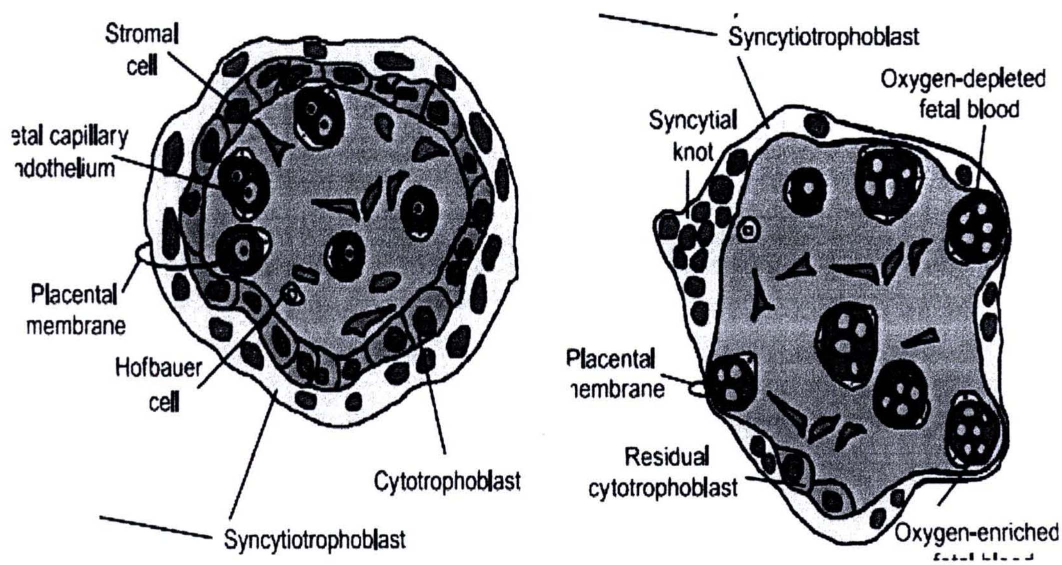
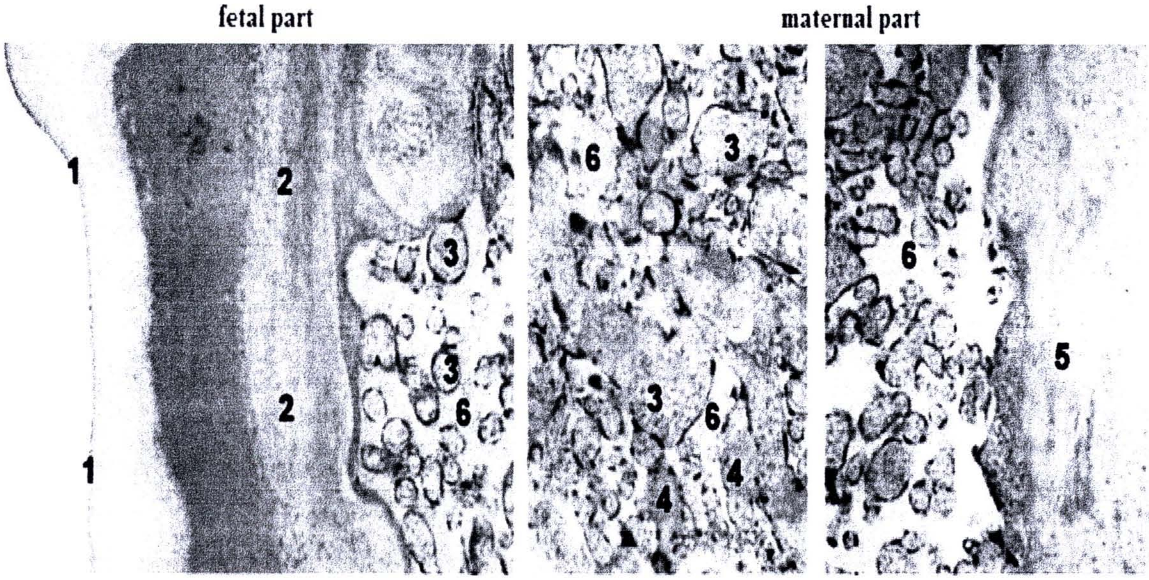


Figure 10 (A) A section through the chorionic villus at approximately 10 weeks and (B) the chorionic villus at full term.

Source: Gudea, et al., 2004

Histology of placental tissues

The placenta composes of fetal and maternal portions, The fetal portion consists of the chorionic plate which forms a part of the wall of the amniotic cavity, and the chorionic villi which arise from the plate. All villi contain fetal blood vessel which are continuous with those in chorionic plate and umbilical cord the intervillus space. Between villi is filled with circulating of maternal blood which nutrients are taken into the fetal blood within villi and then transported to the fetus. The villi which attach to the maternal part are referred to as anchoring villi. The maternal part of placenta is formed by decidual basalis. The decidual tissue projected toward the chorionic plate which is known as placental septa as represented in Figure 11 (Zhang SX. 1998).



1 - amnion
2 - chorionic plate
3 - chorionic villi
4 - placental septum (excrescence from decidua basalis)
5 - decidua basalis
6 - blood lacunas

Figure 11 Fetal and maternal portions of the placenta

Source: http://www.histol.chuvashia.com/atlas-en/female-04-en.htm

Chorionic villi

Chorionic villi consist of connective tissue core and the surrounding trophoblast layer. Early human placenta, the trophoblast is composed of two layers. The inner layer is cytotrophoblast, and outer is syncytiotrophoblast. The cytotrophoblast is made up of a layer of large, ovoid, discrete cells with a pale-stained cytoplasm and spherical nucleus. The cytotrophoblast is separated from core connective tissue by a thin basement membrane. The syncytiotrophobast is a dark layer of cytoplasm without distinct intercellular boundary. It contains numerous small, ovoid nuclei. The cytoplasm is basophilic because of abundant granular endoplasmic reticulum as well as numerous lysosomes. In late human placenta, in the chorionic villi is the disappearance of the cytotrophoblast layer. The core of chorionic villi is a loose connective tissue derived from the extraembrionic mesenchyme. It posses some collagen and mesenchymal cells, the fibroblast that connect with other through their process. The hofbauer cells, a kind of macrophage, are also present in connective tissue. These cells are large, and have big, round nucleus and phagocytic vacuoles within the cytoplasm. The fetal capillaries in the core lined with typical endothelium (Zhang, 1998) (Figure 12).

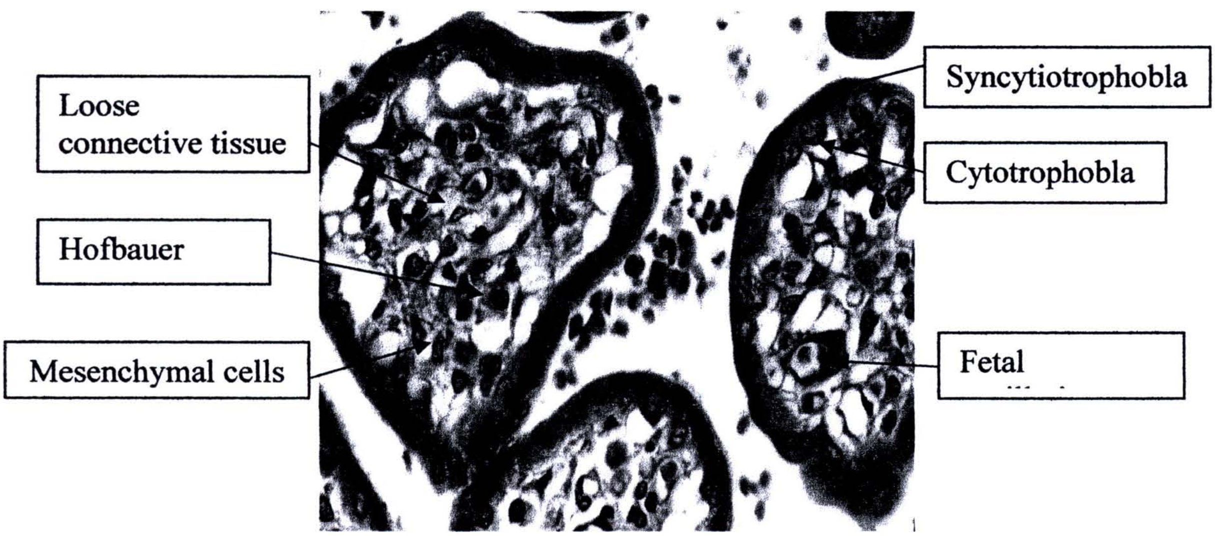


Figure 12 Chorionic villous

Source: modified from http://www.histol.chuvashia.com/atlas-en/female-04-en.htm

Decidual cell

The decidual cells are derived from the stromal cells of the endometrium. At a late stage if pregnancy, the decidual cells enlarge and are separated by the intercellular matrix. These cells are ovoid, and have a large nucleus with a prominent nucleolus. The cytoplasm appears basophilic, because it contains a great amount of rough endoplasmic reticulum. The decidaul cells are separated from the very thin syncytiotrophoblastic layer by a thick layer of fibrinoid within which some decidual cell and fibroblasts may be found (Zhang SX. 1998) (Figuer 13)

Figure 13 Decidual cells

Source: http://www.bu.edu/histology/p/19905ooa.htm

Placental functions

The main functions of placenta can be classified under manipulation of transport, metabolism, protection and endocrine. The placenta acts as fetal lungs (gaseous transfer), gastrointestinal tract (uptake of nutrients), and kidneys (control of fluid volume and removal of waste metabolites) while these organs are generated. It also acts as an endocrine organ, discharging steroid and peptide hormones (Carlson, et al., 1999; Gude, et al., 2004).

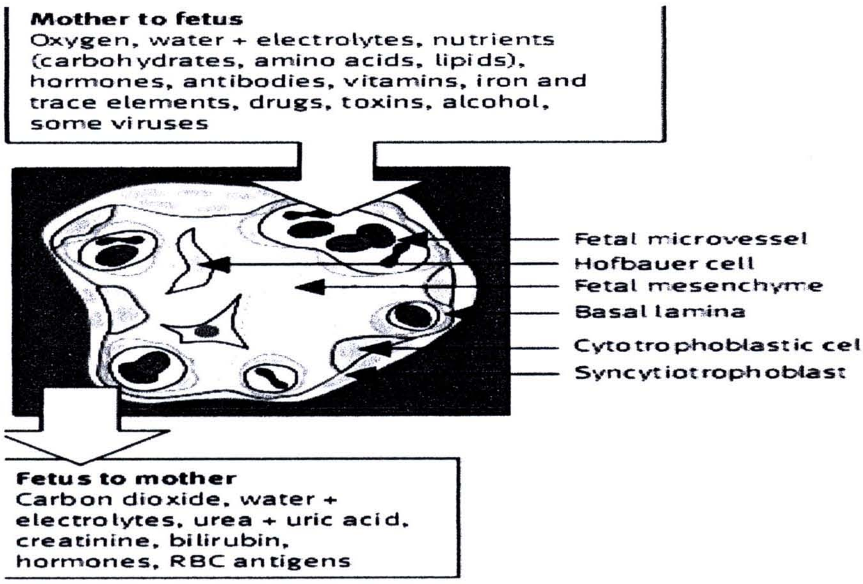


Figure 14 Exchange of substances across the placenta

Source: Leo Donnelly and Gillian Campling 2008

1. **Placental transport and metabolism**

1.1 Glucose

Glucose is the main carbohydrate transported across the placenta from mother to fetus. The process is mediated by the GLUT (Glucose transporter) family of transporters, especially the GLUT-1 isoform, which occurs within maternal (microvilli) and fetal (basal) membranes of the syncytiotroblast. Other isoforms occur at different time of gestation (GLUT-3 was found in endothelium cell lining the fetal capillary, GLUT-4 presented in placental stromal cell and GLUT-12 was found in villous vessel smooth muscle cells and villous stromal cells at term placenta whereas some letter reported that these transporters may be a role in first trimester of placenta) (Young and Heath, 2000; Donnelly and Campling, 2008; Carlson, et al., 1999; Gude, et al., 2004).

1.2 Amino acids

The high rate of protein synthesis needed for growth and development means that fetal concentrations of amino acids are generally higher than the maternal by active transport mechanisms in the microvillous or basal syncytiotrophoblast membranes. These mechanisms include Na+-dependent as well as Na+/Cl−-dependent, cationic and glycoprotein-associated amino acid transporters. The transporters may be inhibited by alcohol or nicotine (Young and Heath, 2000; Donnelly and Campling, 2008; Carlson, et al., 1999; Gude, et al., 2004).

1.3 Fatty acids

Essential fatty acids are a requirement for cellular growth and metabolism. Maternal lipoproteins may be taken up by the placenta via lipoprotein receptors or scavenger receptors The free fatty acids (FFA) that are produced diffuse across the membrane, uptake is probably mediated by fatty acid binding proteins (FABPpm).Within the syncytial cytoplasm, FABP-bound FFA and transported to the fetal vasculature by FATP (fatty acid transport proteins) or diffusion (Young and Heath, 2000; Donnelly and Campling, 2008; Carlson, et al., 1999; Gude, et al., 2004).

1.4 Minerals

1.4.1 Calcium

The fetus accumulates 25–30 g of calcium over the course of pregnancy to mineralize the skeleton (particularly during the third trimester) as well as to contribute to many cellular functions, including cellular growth, neurotransmitter release and signal transduction. L-type voltage-gated calcium channel and transient receptor potential channels as candidate for actively transporting calcium channels in the syncytiotrophoblast membranes (Young and Heath, 2000; Donnelly and Campling, 2008; Carlson, et al., 1999; Gude, et al., 2004).

1.4.2 Iron

Ferritins and transferrins have developed, to store and transport iron which essential elements in many cellular processes, but also a catalyst for the production of harmful reactive oxygen species. Diferric transferrin binds to a receptor on the microvillous syncytiotrophoblast membrane and the complex is internalized into acidic vesicles. Subsequent transport to the fetal vasculature probably involves a divalent metal transporter-1, iron-regulated transporter-1 and copper oxidase (hephaestin) (Young and Heath, 2000; Donnelly and Campling, 2008; Carlson, et al., 1999; Gude, et al., 2004).

1.4.3 Other minerals

Potassium, magnesium and phosphate are all transported across the placenta actively, whereas at least in some species sodium and chloride transfer may occur passively. The situation is quite complex, however, as there are many active ion-transporting systems in the placenta, including Na/K ATPase, Ca ATPase, Na/H exchangers (Young and Heath, 2000; Donnelly and Campling, 2008; Carlson, et al., 1999; Gude, et al., 2004).

1.4.5 Vitamins

Vitamins are transferred from the maternal to the fetal circulations, similarly mineral transport (i.g. iron dissociates from transferrin at the placental interface, and is transported across the placenta)(Young and Heath, 2000; Donnelly and Campling, 2008; Carlson, et al., 1999; Gude, et al., 2004).

2. Placenta fluid regulation

Water derived from the maternal circulation moves between the placenta and the amniotic fluid. Amniotic fluid resorption occurs by fetal swallowing, but also more directly across the amnion to the fetal circulation (The intramembranous pathway). Movement of water across the placental syncytiotrophoblast is dependent upon combination of osmotic and hydrostatic forces, possibly regulated by the expression of aquaporin water channels in the plasma membranes (Young and Heath, 2000; Donnelly and Campling, 2008; Carlson, et al., 1999; Gude, et al., 2004).

3. Placental waste metabolites elimination

Toxic ammonia from purine and amino acid catabolism is converted into uric acid and urea, are diffuse passively across the syncytiotrophoblast. Bilirubin is a breakdown product of normal haem catabolism and its unconjugated form is believed to cross the placenta by passive diffusion. Some evidence, carrier-mediated transport may occur at both the microvillous and the basal membranes (Young and Heath, 2000; Donnelly and Campling, 2008; Carlson, et al., 1999; Gude, et al., 2004).

4. Placental hormone synthesis and secretion

The placenta functions as an endocrine organ. It secretes both peptide and steroid hormones, which act to maintain pregnancy. The two main peptide hormones secreted by syncytiotrophoblast are human chorionic gonadotrophin (hCG; produced by the syncytiotrophoblast) and human placental lactogen (hPL), also known as human chorionic somatomammotropin (Gude, et al., 2004). Human chorionic gonadotrophin (hCG) is a glyco-protein hormone. Its role is to prevent the decompose of the corpus luteum of the ovary and maintain progesterone production. After 6–8 weeks the placenta takes over the production of progesterone and continues to secrete smaller amounts of hCG (Gude, et al., 2004). hPL acts to promote the growth of breast tissue in preparation for lactation and also has metabolic effects. It decreases maternal insulin sensitivity, and raises maternal blood glucose levels that helps ensure adequate fetal nutrition (Young and Heath, 2000; Donnelly and Campling, 2008; Carlson, et al., 1999; Gude, et al., 2004). The major steroids secreted by the placenta are estrogens and progesterone. Progesterone which maintains the endometrium reduces myometrial activity and suppresses maternal immunological responses to fetal antigens. It acts as a precursor for steroid production by the fetal adrenal glands. Production of estrogens involves the use of androgenic substrates from the mother and fetus (Young and Heath, 2000; Donnelly and Campling, 2008; Carlson, et al., 1999; Gude, et al., 2004).

5. Gas exchange across the placenta

The placental membrane is highly penetrative to respiratory gases by flow-limited passive diffusion. The diffusion of oxygen (O_2) can occur rapidly from maternal to fetal blood, and of carbon dioxide (CO_2) from fetal to maternal blood. The placental blood-blood barrier is much thicker than the blood-gas barrier of lung. Thus the fetus compensates by having an increased of fetal hemoglobin concentration (HbF); 170 g/liter in fetus against 120 g/liter in mother. Interestingly, fetal hemoglobin has a higher affinity for oxygen and a lower affinity for carbon dioxide than maternal hemoglobin. This will therefore favors transfer of oxygen to the fetus and carbon dioxide to the mother (Young and Heath, 2000;0 Donnelly and Campling, 2008; Carlson, et al., 1999; Gude, et al., 2004).

6. Placental immunology

The placenta generally forms a barrier against transmission of many bacteria from mother to fetus. However, some bacteria, some protozoa, and a number of viruses can be transmitted across the placenta. For example, although the majority of human immunodeficiency virus (HIV) infection is transmitted from mother to baby around the time of birth, transplacental HIV transfer may occur, e.g. via HIV binding to lectins expressed by the placenta with subsequent viral absorption. Other viruses that can infect the fetus include cytomegalovirus, rubella, polio, varicella, variola and coxsackie viruses. The bacterium that causes syphilis can also be transmitted across the placenta, as can the protozoal parasite that causes toxoplasmosis. It has also been postulated that viral infection of trophoblast may be related to poor pregnancy outcomes (Young and Heath, 2000; Donnelly and Campling, 2008; Carlson, et al., 1999; Gude, et al., 2004).

Divalent metal transporter-1 (DMT-1)

Divalent metal transporter-1 (DMT-1) has four names as defined in Table 1. It was first identified in 1995, in a screen for homolog of NRAMP1, as a protein involved in host defense. In the last few years, the family of natural resistance-associated macrophage protein (NRAMP) metal ion transporters has been reported and considered to play a role in metal ion homeostasis (Forbes et al., 2001). Members of the NRAMP family share remarkable protein sequence identity of yeast, plant, fly with mammalian protein. This family is represented in mammals by NRAMP1 and NRAMP2 as shown in Table 2.

Table 1 Multiples name of DMT-1 transporter

NRAMP 2	natural resistance-associated macrophage protein 2
DCT1	divalent cation transporter-1
DMT1	Divalent metal transporter-1
SCL11A2	solute carrier family 11, member 2

Source: Garrick et al., 2003

Table 2 Discover of NRAMP family members

Gene name	Gene expression	Functions	References
SMF1, SMF2, SMF3 (suppressor of *mifl*)	Yeast	Unknown	Gunshin, et al., 1997
MVL (*malvolio*)	Fly	-	Gunshin, et al., 1997
NRAMP1	Mammals	Metal transport across the phagosomal membrane of macrophages	Gunshin, et al., 1997; Nevo and Nelson, 2006
NRAMP2		General divalent metal-ion transporter at the plasma membrane of cells of both the duodenum and in peripheral tissue	Gunshin, et al., 1997; Nevo and Nelson, 2006

Major function of DMT-1

In iron metabolism, there are three critical steps in which NRAMP2 may function as an iron transporter: (i) the apical membrane dietary iron transport step in intestinal enterocytes, (ii) the phagosomal iron transport step from phagocytosed red blood cells in reticuloendothelial systems, and (iii) the endosomal iron transport step in the Tf cycle in erythrocytes and other cells (Hergaux, et al. 1999).

Role of DMT-1 iron uptake via the enterocyte

A previous model of DMT-1 iron uptake in the enterocyte as shown in Figure 15, the majority of dietary non-heme iron entered the gastrointestinal tract in the ferric form. The ferric iron (Fe^{3+}) must be converted to ferrous form (Fe^{2+}) by ferri-reductase at the brush border membrane, possibly a duodenal cytochrome b (Dcytb) (McKie, et al., 2001). The ferrous ion was transported across the brush border membrane via DMT-1 into the enterocyte (Nemeth, et al., 2004). DMT-1 is required for intestinal uptake of dietary non-heme iron but not essential for maternal iron transfer across the placenta to the fetus (Gunshin, et al., 2005). The intracellular step of iron absorption refers to the intracellular distribution of the transported iron to the basolateral surface or to iron binding proteins (e.g., heme, nonheme iron binding proteins, and ferritin) in the enterocyte. The mechanism of intracellular iron transport is unknown. Finally, iron is transferred across the basolateral membrane of the enterocyte by the iron exporter ferroportin 1 or Metal transporter protein 1 (MTP1) (McKie, et al., 2000). There, the ferrous iron was oxidized to ferric form by hephaestin (Vulpe, et al., 1999). Hepcidin binds to plasma membrane-bound ferroportin 1, is known to regulate iron release from cells such as enterocytes. (Nemeth, et al., 2004).

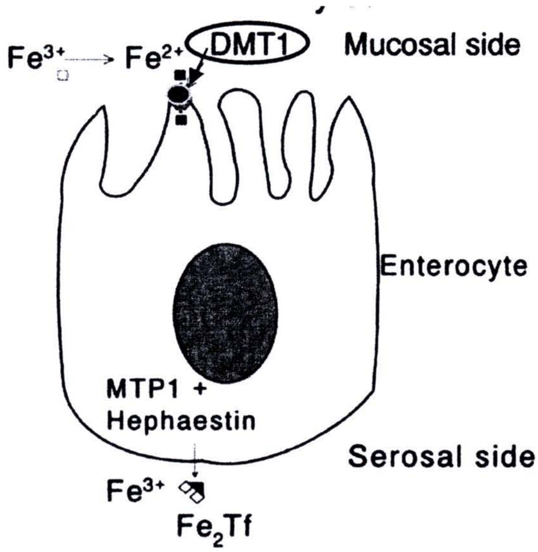


Figure 15 Function of DMT-1 in enterocytes.

Source: Garrick, et al., 2003

Role of DMT-1 iron transport in endosome

In reticuloendothelial systems, macrophages play a role in iron metabolism by phagocytosis of red blood cells, breaking down their hemoglobin, and recycling iron to transferrin (Tf) (Andrews, 1999). The bulk of iron used for erythropoiesis has passed through this recycling pathway. In reticuloendothelial macrophages, an iron transporter is needed in the phagosomal membranes to release the phagosomal free iron into the cytoplasm. These cells specifically express Nramp1, which is localized to the late endosomes and lysosomes and which is associated with phagosomal membranes during phagocytosis (Andrews and Levy, 1999). Gruenheid and coworkers (Gruenheid, et al. 1999) reported that Nramp2 is also expressed in macrophages and associated in this pathway. In the first step, plasma Fe^{3+}-Tf attaches to specific Tf receptors on the cell surface. The Tf-TfR complexes are then internalized within clathrin-coated vesicles. Iron is released from the Tf within the endocytic vesicles that involves endosomal acidification. Influx of protons into the endosomes occurs via an ATP-dependent proton pump. At the acidic pH of endosomes, iron is released from Tf, and the resulting apo-Tf remains bound to its receptor in the acidic endosomes (Dautry, et al. 1983; Rao, et al 1983). The apo-Tf-TfR complex is rapidly sorted to the recycling endosomes and moves back to the cell surface. The free Fe^{3+} released to endosomes and is reduced to Fe^{2+} by oxidoreductase (Nunez et al. 1990). Finally, free Fe^{2+} is transported to late endosomes and lysosomes and is then transported into the cytoplasm by NRAMP2 as shown in Figure 16 (Tabuchi, et al. 2000).

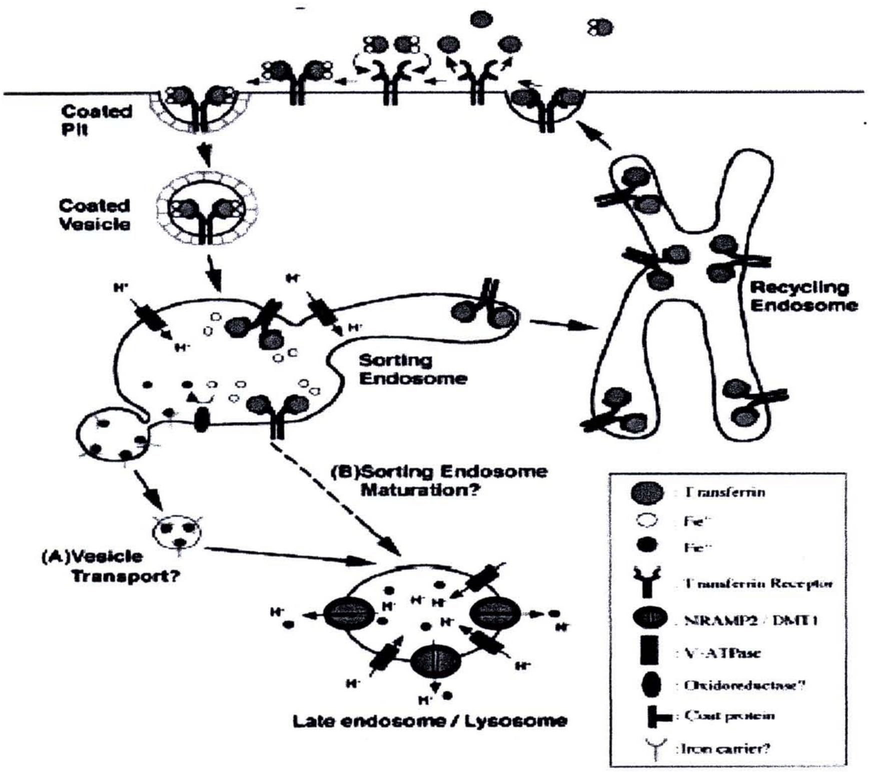


Figure 16 DMT-1 in endosome and transferring cycle

Source: Tabuchi, et al., 2000

Iron distribution in human

The human body contains approximately 3–5 g of iron, represented as Figure 17. The majority of body iron (~60–70%) was utilized within hemoglobin in circulating red blood cells (Andrews, 1999; Ponka, 1997). Other iron-rich organs are the liver and muscles. Approximately 20–30% of body iron is stored in hepatocytes and in reticuloendothelial macrophages, within ferritin. The remaining body iron is primarily localized in myoglobin, cytochromes, and iron containing enzymes. A healthy individual absorbs daily 1–2 mg of iron from the diet, which compensates nonspecific iron losses by cell desquamation in the skin and the intestine. In addition, menstruating women physiologically lose iron from the blood. Erythropoiesis requires approximately 30 mg iron/day, which is mainly provided by the recycling of iron via reticuloendothelial macrophages. These ingest senescent red blood cells and release iron to circulating transferrin. The pool of transferrin- bound iron (~3 mg) is very dynamic and undergoes >10 times daily recycling.

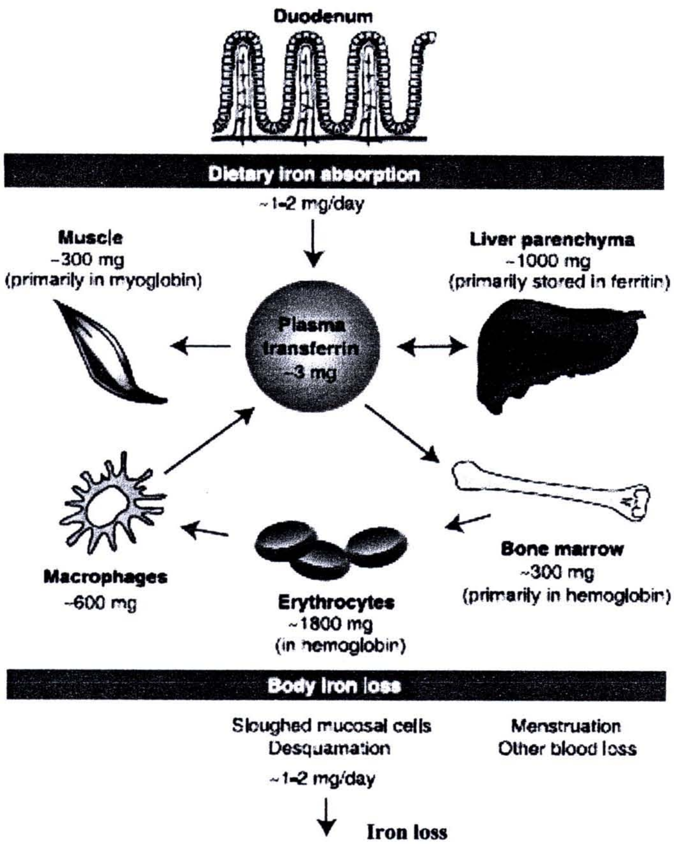


Figure 17 Iron distributions in the adult human body

Source: Papanikolaoua and Pantopoulos, 2005

Regulation of cellular iron metabolism: the IRE–IRP system

The cell must regulate all stages of iron metabolism including uptake, storage, and utilization. In vertebrates, expression of proteins such as TfR and ferritin are regulated by cellular iron levels at the posttranscriptional level (Hentze MW.and Kuhn, LC., 1996). Briefly, mRNAs of some proteins of iron metabolism contain Iron response elements (IREs) in the untranslated regions (UTRs). IREs are 30-nucleotide hairpin structures that are conserved in vertebrates and some insects and bacteria. Under iron-starved conditions, iron regulatory protein 1 (IRP1) and iron reguratory protein 2 (IRP2) bind to the IREs. IRP1 and IRP2, function as intracellular iron sensors (Hentze, et al., 2004; Pantopoulos, 2004). IRP2 is regulated in response to iron and oxygen supply by distinct post-translational mechanisms (Hentze, et al., 2004; Pantopoulos, 2004). In mRNAs for proteins involved in iron uptake such as TfR, the IREs are found in the 3′UTR; IRP binding to IREs in the 3′UTR stabilizes the mRNA increasing translation of the proteins. Proteins such as ferritin that are involved in sequestering iron have IREs in the 5′UTR; IRP binding to 5′IREs results in translational inhibition and decreased synthesis of the proteins. RNA binding activity of IRP1 is regulated by the formation of an Fe-S cluster which inhibits IRE binding in iron replete conditions (Haile, DJ., et al., 1992).The IRE/IRP regulation of iron metabolism has been reviewed recently (Pantopoulos, K., 2004; Rouault, TA., 2006). In addition, the mRNAs encoding the iron transporters DMT-1 and ferroportin 1 contain an incompletely characterized IRE in their 3' UTR. Iron levels are regulated by iron regulatory proteins (IRP) in most cells and tissues, by IREs binding on either the 3′-end or the 5′-end of the mRNA. Principally, binding to the 3′-end stabilizes the mRNA and increases translation, whereas binding to the 5′-end inhibits translation (e.g. the transferrin receptor IRE is located at the 3′-end, whereas that of ferritin is at the 5′-end) as shown in Figure 18. This is not continually the case, although the IRE for IREG1 is at the 5′-end, and yet translation is increased in iron deficiency in the gut (McKie, et al., 2000). Although, this IRE only marginally mediates iron regulation in cultured cells (Gunshin, et al., 2001) and its mechanism of action is not well understood (Henze, et al 2004). The situation is more complex, as a 5' promoter/exon 1A region actswith the 3' IRE-containing terminal exon to control DMT-1 expression (Hubert and Hentze, 2002).

A.

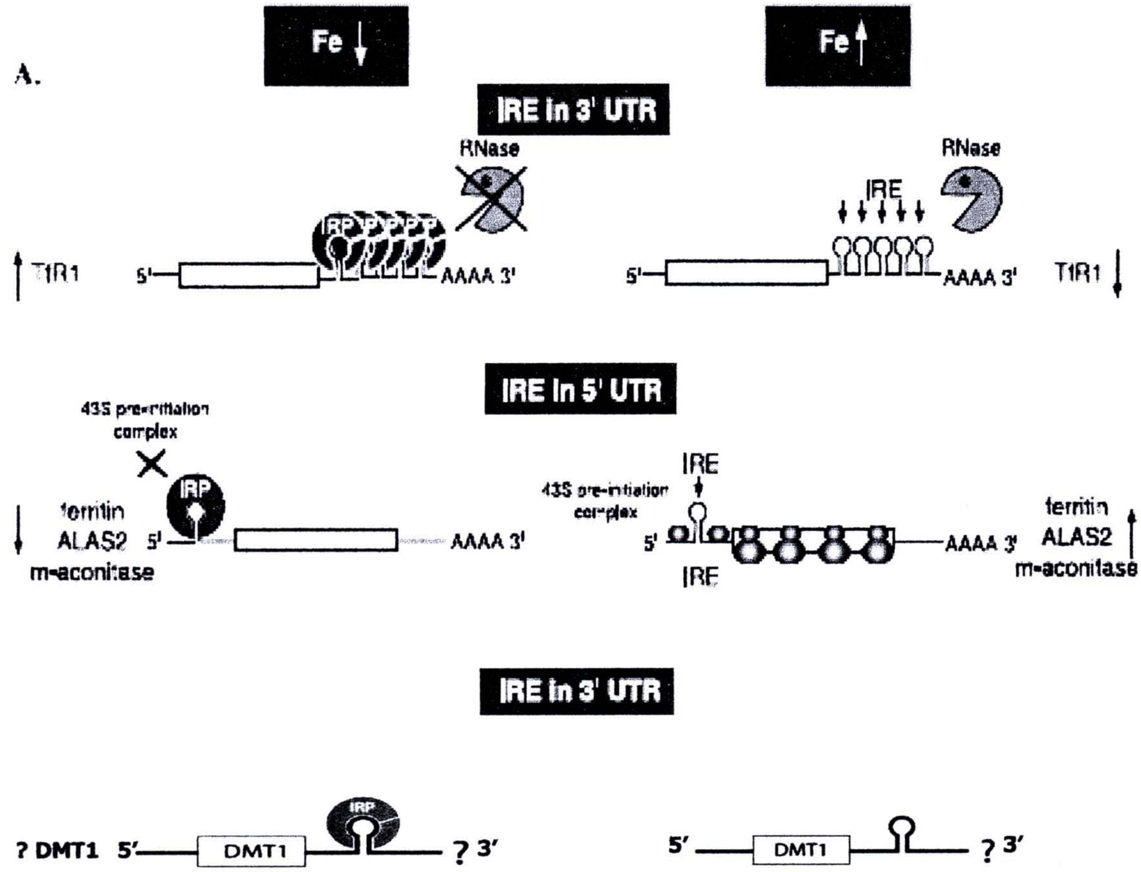


Figure 18 Homeostatic responses to iron by IRE–IRP interactions of TfR, ferritin and DMT-1 transporters

Source: Modified from Papanikolaoua and Pantopoulos, 2005; Henze, et al., 2004

Multiple functions of DMT-1

DMT-1 transports a wide range of divalent metals-ions including Fe^{2+}, Mn^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Pb^{2+}, Zn^{2+} and Cd^{2+} (Gunshin, et al., 1997; Conrad, et al. 2000; Garrick and Dolan, 2002; Elisma and Jumarie, 2001; Olivi, et al., 2001). The information on these metals are potentially transported by DMT-1 and is summarized in Table3.

Table 3 DMT-1 interaction with metals

Metal	Conductance [a]	Uptake [b]	Competition [c]	Tissue/mutation [d]
Fe^{2+}	Yes	Yes	Yes	Yes
Fe^{3+}	No	No	No	NA
Zn^{2+}	Yes	No	No	No
Mn^{2+}	Yes	Yes	Yes	Yes
Ni^{2+}	Yes	Yes	Yes	Untested
Co^{2+}	Yes	Yes	Yes	Untested
Cu^{2+}	Yes	Untested	Yes [f]	No
Cd^{2+}	Yes	Untested [e]	Yes [g]	Untested
Pb^{2+}	Yes	Untested	Yes	Untested

[a] In the *Xenopus* oocyte expression assay (Gunshin, et al. 1997).

[b] In the HEK293T cell expression assay (Conrad, et al. 2000; Garrick and Dolan, 2002; Garrick, M.D., et al.; unpublished data).

[c] Competes with $^{54}Mn^{2+}$ uptake in the HEK293T cell expression assay (Conrad, et al., 2000; Garrick and Dolan, 2002; Garrick, M.D., et al.; unpublished data).

[d] Deficient in liver of *mk/mk* mice (Conrad, et al., 2000 – only tissue tested) or multiple tissues of *b/b* rats (Garrick, et al., 1997 for Fe; and Garrick, M.D., et al.; unpublished data unpublished data for Mn).

[e] Elisma and Jumarie (2001) detect DMT-1 dependent $^{109}Cd^{2+}$ uptake in Caco-2 cells at pH 5.5 (using Fe^{2+} competition as a criterion).

[f] Although Conrad, et al. (2000) observed little competition with K562 cells

[h] Olivi, et al. (2001) also used HEK293 cells for ectopic expression of DMT1 to see a stimulation of $^{109}Cd^{2+}$ and $^{55}Fe^{2+}$ uptake.

Source: Modify from Garrick, et al., 2003

In addition, previous finding represented the affinity ranking of DMT-1 for metal ions transport as Mn > Cd > Fe > Pb ~ C~ Ni >Zn (Garrick MD. et al. 2006). These studies showed that the Km of Mn^{2+} and Fe^{2+} for DMT-1 were ~1 μM and ~3 μM, respectively (Garrick MD. et al. 2006). DMT-1 had a lower affinity of Fe^{2+} than Cd^{2+}, the *K*m value of DMT-1 for Fe^{2+} was approximately 2 μM at pH 5.5 in mammalian cells (Gunshin, H., et al 1997). The averaged *K*m value of DMT-1 for Cd was 1.04 ± 0.13 μM (Okubo, M., et al 2003). DMT-1 acts as a symporter of metals and H^+, while the proton gradient is the driving force for the metal transport, one proton per divalent cation as shown in Figure 19 (Gunshin, et al., 1997; Andrews, et al., 1999; Garrick, et al., 2003).

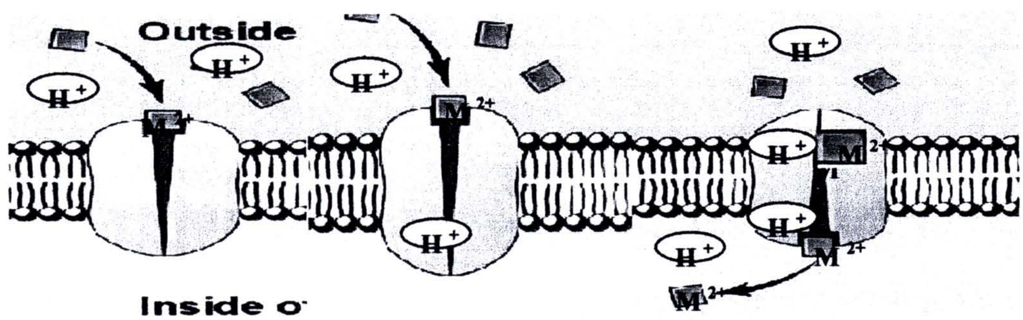


Figure 19 DMT1 involved in the transport of protons and metal-ions

Source: Modify from Nevo and Nelson, 2006

Structure properties of DMT-1

Alignment of the amino acid sequences of the human DMT1 protein identifies a common and conserved hydrophobic core of 10 transmembrane domains and either two non-conserved therefore DMT1 gene consists of 17 exons spread over more than 36 kb. The location of the loop between transmembrane domains 7 and 8 is substantial extracellular loop. Mutations of transmembrane domain 4 have been shown to interfere with DMT1 protein function. Both amino-(N) and carboxy-(C) termini are predicted to be within the cytoplasm. There are four different splice forms, encoding alternative carboxy-termini and alternative 3' untranslated regions. Isoform I has an iron responsive element (IRE) in the 3' untranslated regions (+IRE), similar to IREs was found in the 3' untranslated region of the transferrin receptor mRNA, whereas isoform II lacks IRE in the 3' untranslated regions (-IRE). The two isoforms differ by levels of expression in the various tissues (Andrews, et al., 1999). And, isoform I and isoform II have different C-terminus, of 18-25 amino acid, respectively (Nevo, Y. and Nelson, N., 2006). This suggested that DMT1 protein expression may be controlled post-transcriptional and translation by intracellular iron concentration. Alternative 5′ exons designated 1A and 1B have reported by Hubert and Hentze, 2002. The exon 1A isoform adds an in frame AUG translation initiation codon extending the DMT-1 ORF (open reading frame) by a conserved sequence of 29-31 amino acids (Nevo Y. and Nelson, N., 2006). The alternative splicing at 5’ and 3’ UTR of DMT-1 isoforms are shown in Figure 20.

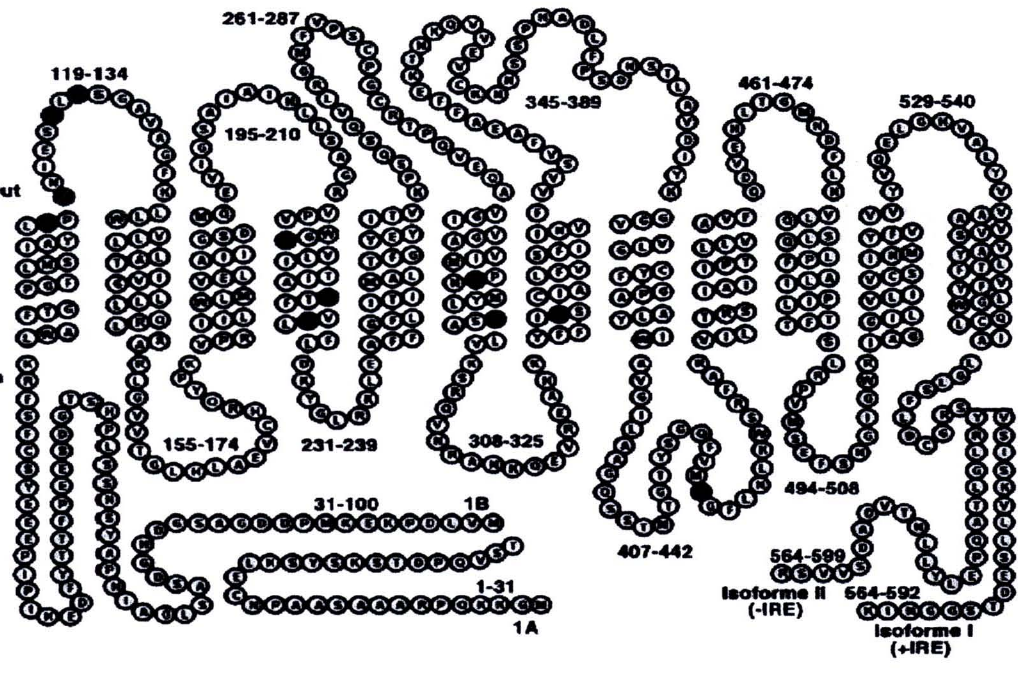


Figure 20 Schematic represent of DMT-1 isoforms including isoform I has iron responsive element (IRE) in 3’ UTR, whereas in 3’ UTR of isoform I has likely lacking iron responsive element (IRE) and alternative 5’ exons designated DMT-1 exon 1A and 1B. (membrane; yellow, intracellular; In, extracellular; Out)

Source: Nevo and Nelson, 2006

Remarkably, the DMT-1 1A mRNA was most expressed in duodenal and kidney cells that was polarized (Hubert and Hentze, 2002). The polarized cells, DMT-1 was localized in apical membrane of cells (Hergaux, et al 1999). On the other hand, the distribution of the DMT-1 1B showed correlated with function of DMT-1 in iron releasing from endosome (Tabuchi, et al., 2000). The predominance of DMT-1 non-IRE expression in epithelial cell lines and DMT-1 non-IRE expression in erythroid precursor cells suggest that DMT-1 acts as apical and endosomal iron transporter (Gunshin, et al., 1997). The DMT-1 IRE increased in rat iron deficiency (Hergaux, et al., 1999). The data consisted that +IRE form as a major role in apical iron transporter (Garrick, et al., 2003). The DMT-1 non-IRE expression in kidney had more than in the intestinal tracks (Hubert and Hentze, 2002). In murine reticulocytes contain a greater fraction of DMT-1 non-IRE than DMT-1 IRE (Hergaux, et al 2001). This evidence supports that the –IRE isoform is usual transporter for iron exit from endosome. The expression of four DMT-1 isoforms in several organ tissues was shown in Table 4. The mRNA expression levels of 173 human solute carrier (SLC) transporters were investigated in adult human tissues (adrenal gland, bladder, bone marrow, brain, colon, heart, kidney, liver, lung, mammary gland, ovary, pancreas, peripheral leukocytes, placenta, prostate, retina, salivary gland, skeletal muscle, small intestine, smooth muscle, spinal cord, spleen, stomach, testis, thymus, thyroid gland, trachea, and uterus). Among them, SLC11A2 was also investigated by real-time reverse transcription PCR using an Applied Biosystems 7500 Fast Real-Time PCR System. The relative expression of each mRNA was calculated by the ΔCt method, where ΔCt is the value obtained by subtracting the Ct value of PPIA mRNA from the Ct value of the target mRNA. These data were shown in Figure 21 (Nishimura and Naito, 2008).

Table 4 Expression of four DMT-1 isoforms in several species

DMT-1 isofroms	Species	Organ tissues expression	Functions	References
DMT-1 IRE	Mouse	heart, kidney*, liver, lung, spleen, thymus, testis*, , esophagus, stomach, duodenum*, jejunum, ileum	Fe regulation	Hubert and Henze, 2002
			Fe and Cd transportation	Kim, et al 2007
	Rat	placenta	Fe rgulation	Gambling, et al., 2001
	Cells	MES23.5 dopaminergic cells	Fe transportation	Xu, et al., 2008
		human umbilical vascular endothelial cells (ECV304) and rat glioma cells (C6),	Pb transportation	Wang, et al., 2009
DMT-1 nonIRE	Mouse	heart, kidney*, liver, lung*, spleen*, thymus*, testis, esophagus, stomach, duodenum, jejunum, ileum	Fe transportstion	Hubert and Henze, 2002
		lung	Fe transportstion	Ghio, et al., 2003
	Cells	Human endothelial cell lines	Fe uptake & transportation	Carraway, et al., 2006
DMT-1 exon 1A	Mouse	Kidney*, duodenum*, testis, gastrointestinal	Fe regulation	Hubert and Henze, 2002
DMT-1 exon 1B	Mouse	heart, kidney*, liver, lung*, spleen*, thymus*, testis, esophagus, stomach, duodenum*, jejunum, ileum	Fr transportation (endosome)	Hubert and Henze, 2002

* is high expression

Tissue	SLC9A8	SLC9A9	SLC9A10	SLC9A11	SLC11A1	SLC11A2	SLC12A1	SLC12A2	SLC12A3	SLC12A4
Adrenal gland	0.0397	0.0349	0.0000214	0.0000099	0.0148	0.00972	0.000109	0.127	0.000140	0.0223
Bladder	0.0266	0.247	0.000248	BLQ	0.00570	0.0115	0.0000803	0.0497	0.000807	0.0928
Bone marrow	0.0181	0.0415	0.000148	BLQ	0.0494	0.0131	0.000405	0.0333	0.00176	0.00657
Brain	0.0147	0.0282	BLQ	0.0000213	0.00244	0.0150	0.000686	0.141	0.000320	0.00586
Colon	0.0166	0.0325	0.0000842	BLQ	0.000261	0.00755	0.0000286	0.293	0.000620	0.0105
Heart	0.0162	0.0612	0.00404	BLQ	0.00156	0.00812	0.0000875	0.0730	0.000253	0.0329
Kidney	0.0169	0.00795	0.000131	BLQ	0.00148	0.0157	1.44	0.0329	0.732	0.0103
Liver	0.00755	0.0454	BLQ	BLQ	0.00263	0.00861	0.000277	0.0114	0.000110	0.00609
Lung	0.0135	0.0561	0.000192	0.0000417	0.0909	0.0142	0.000169	0.0805	0.00109	0.0287
Mammary gland	0.0369	0.00798	0.000177	BLQ	0.000205	BLQ	0.0000291	0.637	0.00108	0.0257
Ovary	0.0150	0.125	0.0000438	0.0000239	0.00124	0.00687	0.0000360	0.0875	0.000112	0.0783
Pancreas	0.0160	0.0103	0.000219	BLQ	0.00190	0.0197	0.0000828	0.0577	0.000970	0.00514
Peripheral leukocytes	0.0260	0.0467	0.0000231	BLQ	0.316	0.00834	0.000468	0.0373	0.00354	0.00682
Placenta	0.0433	0.0797	0.000307	BLQ	0.0209	0.0330	BLQ	0.0227	0.00465	0.0842
Prostate	0.0299	0.0797	0.000231	0.0000135	0.00304	0.0368	0.0000114	0.402	0.000880	0.0225
Retina	0.0283	0.0964	0.000131	0.0000510	0.00476	0.0213	0.00162	0.197	0.000793	0.101
Salivary gland	0.0240	0.0135	0.0000163	BLQ	0.00106	0.0205	BLQ	0.418	0.000780	0.00814
Skeletal muscle	0.0819	0.0638	0.000232	BLQ	0.00105	0.0526	0.000203	0.257	0.000252	0.0808
Small intestine	0.0180	0.0475	0.000118	BLQ	0.000295	0.00852	0.0000110	0.307	0.000877	0.00870
Smooth muscle	0.0202	0.289	0.000217	BLQ	0.00364	0.0143	0.0000070	0.600	BLQ	0.148
Spinal cord	0.0469	0.138	0.000135	0.000146	0.0122	0.0205	0.000710	0.260	0.000930	0.0275
Spleen	0.0266	0.132	0.0000673	0.0000325	0.0226	0.00433	0.0000213	0.0379	0.00657	0.00809
Stomach	0.0308	0.0524	0.0000701	BLQ	0.00350	0.0105	0.0000124	0.339	0.000705	0.0128
Testis	0.0756	0.0178	0.0842	0.0147	0.00146	0.0116	0.00226	0.135	0.00258	0.0275
Thymus	0.0146	0.0725	0.0000543	BLQ	0.00141	0.00820	0.0000239	0.0256	0.00393	0.0100
Thyroid gland	0.0278	0.0288	0.000750	BLQ	0.00338	0.0206	BLQ	0.0698	0.000338	0.0249
Trachea	0.0533	0.0484	0.000146	0.0000951	0.00523	0.0250	0.0000277	1.72	0.00189	0.0212
Uterus	0.0161	0.0451	0.0000426	0.0000057	0.00141	0.0173	0.000388	0.0461	0.000237	0.0315

BLQ, below the limit of quantification. Data are expressed as the ratio of the target mRNA to PPIA mRNA. Experiments were performed in duplicate. The highest values among the various tissues are shown in boxes.

Figure 21 Expression of Human SLC11A2 Transporter mRNAs in Various Tissues

Source: Nishimura and Naito, 2008

Table 5 Localization of DMT-1 protein

Organs	Species	Cellular localization	Functions	References
Duodenum	Mouse and wild type	luminal surface and apical cytoplasm of villus cells, and vascular and lymphatic cells of the lamina propria and in crypts cells. Intracellular and apical side of epithelial cells and little or no brush border of enterocytes	Plasma membrane and intracellular transport of Fe	Hergaux, et al., 2000
	Human	brush border of enterocyte	Transport Fe from the intestinal lumen, uptake of cadmium	Griffith, et al., 2000 and Tallkvist, et al., 2002
	Coco-2 cell	vesicle and late endosome	Fe regulation and delivery to intracllular Fe pool	
Liver	Rat	cell membranes and intracellular of hepatocytes lining the sinusoidal space	Transmembrane Fe transport and endosomal Fe export	Trinder, et al., 2000
Kidney	Rat and mouse	intracellular of late endosomes and lysosomes and subapical membrane of vesicle	Cd^{2+} and Fe^{2+} transport	Smith and Thévenod, 2009
		apical and intracellular in hepatocytes	apical Fe entry	Ferguson, et al., 2001
Mammary gland	Rat	luminal membrane of mammary epithelial cells	endosomal Fe export	Kelleher and Lonnerdal, 2004

Table 5 (Cont.)

Organs	Species	Cellular localization	Functions	References
Placenta	Human	cytoplasm and fetal (basal) membrane of syncytiotrophoplast (STB) and fetal vessels, hofbauer cell	Fe transport (endosome)	Georgieff, et al., 2000
		cytoplasm and plasma membrane of STB	Fe transport	Chong, et al. 2005
		cytoplasm of STB and stromal cells	Fe transport (endosome)	Bastin, et al., 2006
Lung	Mouse	airway epithelial cells and alveolar macrophages	Fe transport	Gicho, el al., 2003
	Rat	airway epithelium, bronchus associated lymphoid tissue (BALT) and macrophages	Mn absorption	Thompson, et al., 2006
Macrophage cell line RAW264.7	Rat	intracellular in macrophage cell	Divalent metals transport	Jabado, et al., 2011
Hippocampus	Rat	Astrocyte at astrocytes end foot	Metal ions transport	Wang, et al., 2002

Association of DMT-1 in several organs

1. Intestine

In 2002, Park and coworkers (Park, et al., 2002) had been reported that DMT-1 can transport Fe and Cd in intestinal, and in animal fed Fe-deficient (FeD) diet had higher Cd concentration and DMT-1 expression than animal fed Fe-sufficient (FeS) diet especially in the duodenum. These results indicated that DMT-1 mRNA is likely increased in intestine by body iron depletion and increase Cd uptake. In addition, several studies had been shown DMT-1 is a transmembrane glycoprotein that is an apical membrane iron transporter in intestinal epithelial cells (Fleming, et al., 1999; Gushin, et al., 1997; Beard and Hanm, 2009).

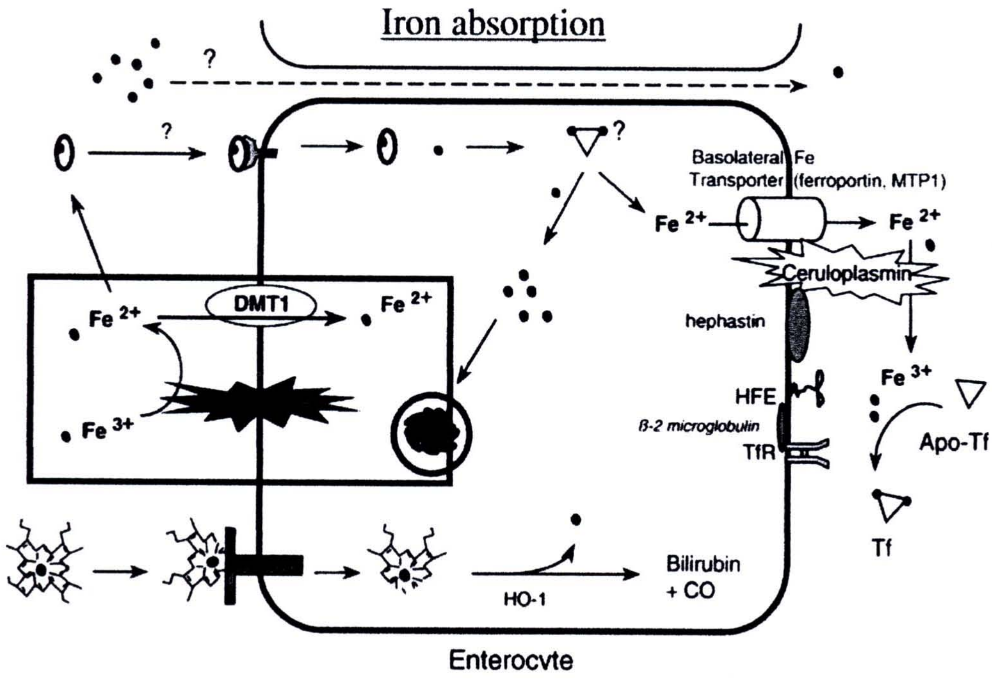


Figure 22 The iron absorption in enterocytes of the upper GI tract

Source: Beard and Hanm, 2009

The majority of dietary iron was explained that iron enters the gastrointestinal tract in the ferric form. The ferric iron must the first be converted to ferrous form by duodenal cytochrome b (Dcytb). The ferrous ion is then transported across the brush border membrane via the DMT-1 into the enterocyte. The intracellular step of iron absorption refers to the intracellular distribution of the newly transported iron to the basolateral surface by the iron exporter ferroportin 1 or to iron binding proteins (e.g., heme, non-heme iron binding proteins, and ferritin) (Beard and Hanm, 2009). The study of effect of Cd on DMT-1 expression in intestinal rat showed that an Fe deficient diet can increased Cd accumulation as well as DMT-1 and MTP-1 expression in the duodenum. However, Cd and DMT-1 and MTP-1 expression returned to control values when rat were ingested Fe sufficient for 4 weeks. These results suggest that DMT-1 and MTP-1 involved in Cd absorption in duodenum rat (Ryu, et al., 2004).

2. Kidney

By immunolocalization, DMT-1 immunoreactivity had been shown in renal cortex whereas less stained in medullary region. It was mainly localized in apical and basolateral membranes of proximal tubule. Moreover, immunogold labeling and electron microscopy techniques were used to pinpoint the intracellular DMT-1 staining to late endosomes and lysosomes. The data implied that DMT-1 was unlikely to be responsible for iron transport across the apical membrane of proximal tubule, and might be involved in movement of iron across late endosomal and lysosomal membrane (Abouhamed, et al., 2006). In animal model study, DMT-1 localization showed the species difference between the kidneys of mice and rats (Canonne, et al., 2002). The hypothetical model was suggested for iron handling by proximal tubule. Then transferring iron is transited to late endosome/lysosome in proximal tubule where the prevailing acidic acid PH results iron to dissociated from transferring and translocated out of the endosome into cytosol via DMT-

1. Iron is storage in cytosol by ferritin and exports to circulation via ferroportin (FPN-1) or MTP-1 (Smith and The'vanod, 2009).

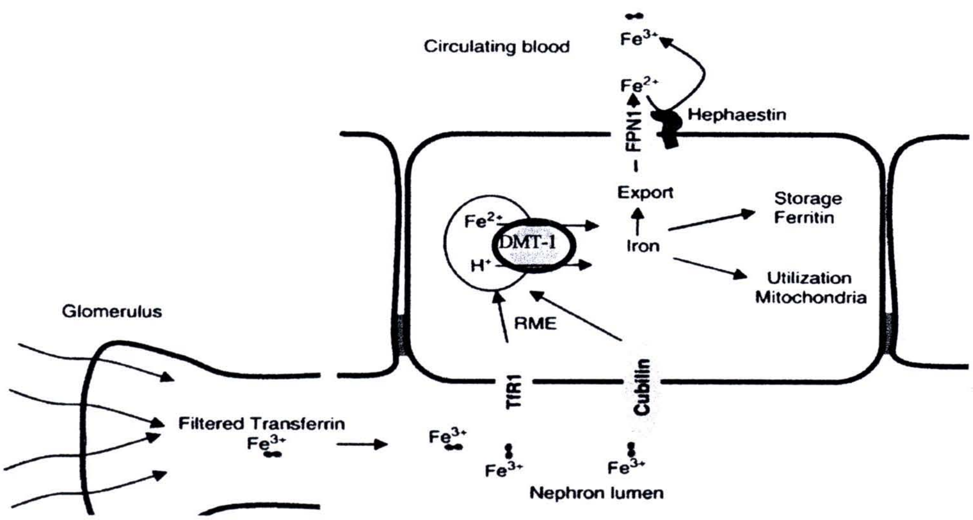


Figure 23 The iron transport in proximal tubule via DMT-1

Source: Smith and The'vanod, 2009

3. Brain

Previous study about DMT-1 mRNA expression in developing rat brain had shown that the two isoforms of DMT-1 mRNA expressed in cerebellum, cortex and hippocampus (Chengwu, et al., 2009). The DMT-1 protein expression had been documented that the DMT-1 protein level in cerebellum cortex and hippocampus was significant the highest in rats fed by Pb and Cd compare with non-fed rats (Chengwu, et al., 2009). According to this result, it implied that Pb and Cd exposure might induce DMT-1 protein synthesis and had implications for transport of toxic metal in developing rat's brain (Gu, et al., 2009). By immunofluorescence technique, it showed that DMT-1 was localized primarily in the region between the nucleus and the apical plasma membrane in rat choroid plexus model (Xueqian, et al., 2008). A hypothetical scheme for brain iron metabolism had been reported since 2007 by Ke Y. and Qian Z.M. as shown in Figure 24. The transferrin bound transferrrin receptor (Tf–TfR) pathway may be the major route of iron transport across the luminal membrane of the blood brain barrier (BBB). Transferrin bound iron (Tf-Fe) is taken up by endothelial cells of BBB. DMT1 may play a role in translocation of iron from endosome to cytosol. However, iron may also transport across the luminal membrane by a DMT-1 mediated progress. Iron (Fe^{2+}) probably crosses the abluminal membrane via ferroportin-hephaestin (FP1–Hp) and/or Hp-independent export systems. Tf-Fe is taken up by neurons and astrocytes via a TfR-mediated process. Whereas, Fe^{3+} is taken up by neurons and astrocytes probably via DMT-1 mediated process, respectively. DMT-1 in astrocytes implies that DMT-1 is likely to mediate iron influx into astrocytes.

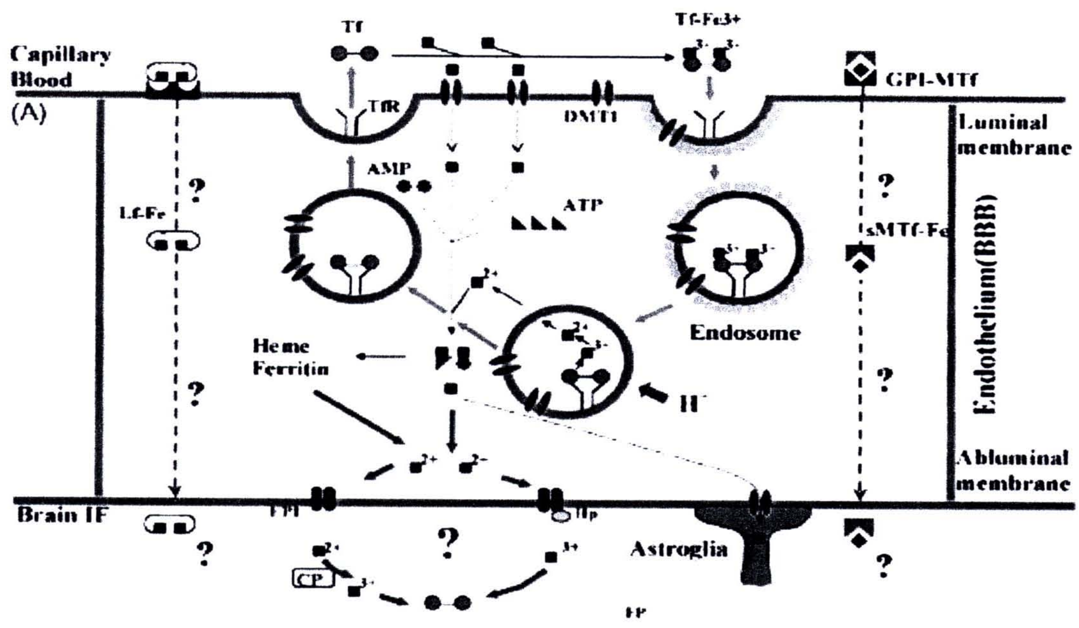


Figure 24 Iron transport across the Blood Brain Barrier (BBB)

Source: Ke and Qian, 2007

4. Lung

The detection of DMT-1 mRNA by *In situ* analysis was found that DMT-1 mRNA expressed in airway epithelium and bronchus-associated lymphatic tissue (BALT). Interestingly, DMT-1 signal was significantly reduced in rats fed by the high iron diet (Thompsona, et al., 2006).

5. Placenta

DMT-1 has been previously described in human placenta (Georgieff, et al., 2000) Immunohistochemistry demonstrated DMT-1 was localized both in cytoplasm of the syncytiotrophoblast cell, at the junction of the fetal membrane of the syncytiotrophoblastic membrane, fetal vessels and the Hofbauer cell. In contrast, TfR was localized predominantly to the maternal (apical) side of the syncytiotrophoblastic membrane (Georgieff, et al., 2000). They proposed the iron transport model in human syncytiotrophoblast as represented in Figure 25.

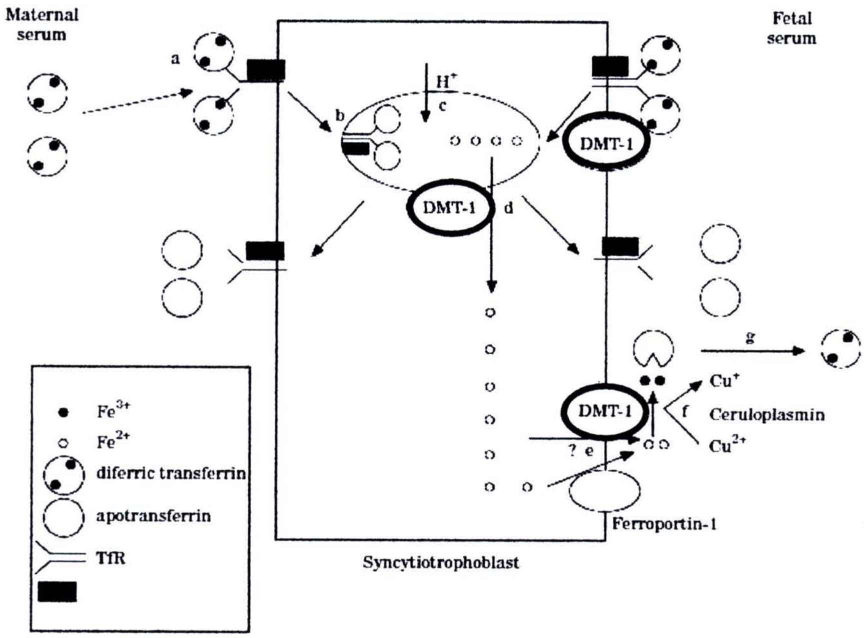


Figure 25 Iron transportation in human syncytiotrophoblast model; Maternal diferric transferrin bounds to apical membrane transferrin receptor (TfR) (a). TfR membrane complex is internalized as an endosome (b) and is subsequently acidified (c) to convert the iron to its ferrous form and to release it from transferrin. The ferrous iron is transported out of the endosome (d) by DMT-1. Whether ferrous iron is then transported across the basal membrane by DMT-1 and ferroportin 1 is unclear (e).

Source: Georgieff, et al., 2000

According to previous study, DMT1 was suggested to be a candidate for transport of iron out of the endosome and across the basal membrane to the fetus. In addition, previous study has been proposed possible model of iron transport from maternal to fetus as shown in Figure 26 (McArdle, et al., 2003; McArdle, et al., 2008). The mechanisms are described in the text. In briefly, Fe-transferrin binds to the receptor, which is internalised into coated vesicles. The iron is released following acidification by DMT-1 to cytosol and transferred across the basal membrane to fetus by Iron regulatory protein 1 (IREG1) and it is oxidized to Fe^{3+} by Cu oxidase which may or may not be the same as hephaestin. (McArdle, et al., 2003)

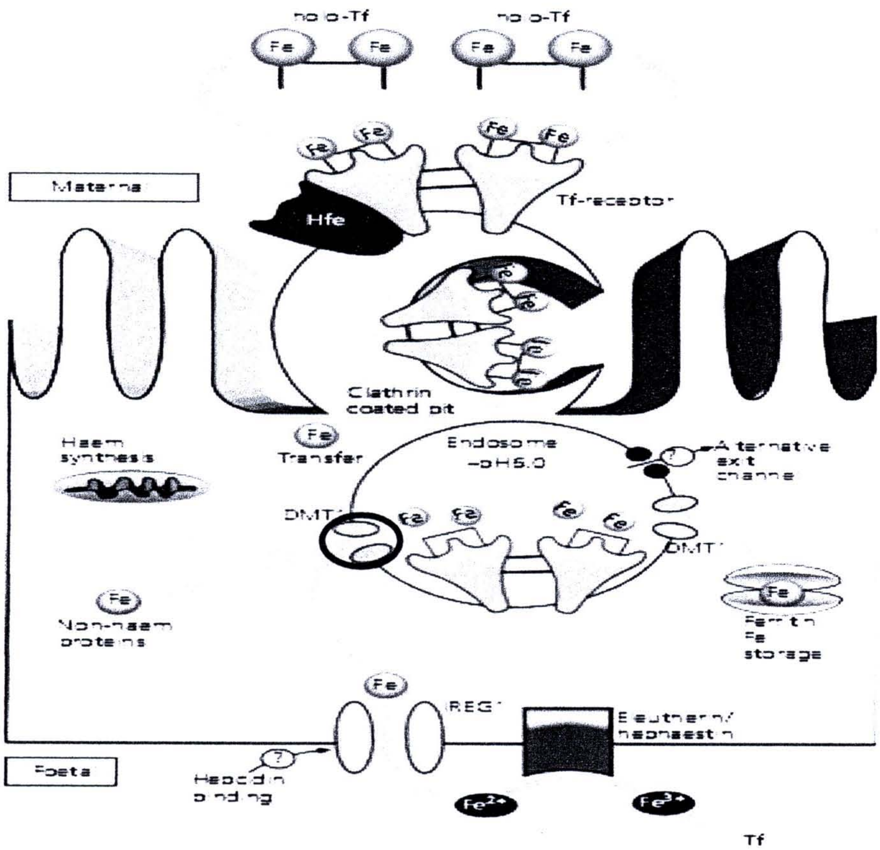


Figure 26 Model of iron transfer across the placenta

Source: McArdle, et al., 2008

Association of Cd and DMT-1 in placenta

DMT-1 mRNA was found in two isoforms; divalent metal transporter 1-iron responsive element (DMT-1 IRE) and divalent metal transporter 1-non iron responsive element (DMT-1 nonIRE). Both isoforms expressed in first trimester human placental tissue. DMT-1 IRE isoform was predominantly expressed in fetal placental tissue (Chong, et al., 2005) according to previous result in adult intestine as described by Hubert and Hentze in 2002. This result suggested a role of this protein isoform in iron-regulatory absorption pathway of divalent metal ion in these tissues (Chong, et al., 2005). The study of placental protein expression related to iron transfer in pregnant rats fed with iron deficient diet showed that TfR and DMT-1 IRE expression were increased at both mRNA and protein levels. In contrast, the non-IRE isoform showed especially increased in protein expression level. These results showed that expression of DMT-1 was up-regulated in maternal iron deficiency (Gambling, et al., 2001). In addition, iron deficiency during pregnancy leaded to increased Cd absorption and Cd body burden (Akesson, et al., 2002). The correlation between Cd absorption and DMT-1 expression were observed in pregnant rat and suggested a role for DMT-1 in the increased absorption of Cd during pregnancy (Leazer, et al., 2002). During pregnancy, essential nutrients requirements increase and the increased demand for essential metal by the fetus also facilitates increased intestinal Cd absorption and subsequent placental transport to the fetus. DMT-1 has been shown immuno-histochemically to localize in human placenta. DMT-1 plays a crucial role for dietary Fe uptake in the duodenum but also recognizes nonessential metals such as Cd. In the previous study, they investigated DMT1 IVS4+44C/A single nucleotide polymorphism (SNP) has an effect on Fe, Zn essential trace element levels and Pb, Cd toxic metals accumulation in the placenta samples. These result represented no significant association between the IVS4+44C/A SNP in the DMT-1 and Pb, Zn, and Fe levels in the placenta samples but statistically significant association was detected with the Cd concentration ($p = 0.001$)

(Kayaalti, et al., 2010). The increasing of Cd can induced light microscopic changes in placenta, female Wistar rats were injected daily with different doses of $CdCl_2$ during the first 19 days of pregnancy. The placentas of highly Cd exposed animals showed reduced weights and amount of collagen in the basal membranes around fetal blood vessels and to the relative volume densities of the fetal blood vessels (Roelfzema, et al., 1985). Also, Cd inhibited placental and trophoblast proliferation though interactions with the intracellular calcium binding protein, calmodulin (CaM). The trophoblast proliferation was a major impact on the institution of alive in pregnancy (Powlin, et al., 1997). In addition, the cord blood Cd was reported that was significantly correlated in smokers, only about 10% of that in maternal blood (Osman, et al., 2000). Multiple linear regression analysis indicated that cord blood Cd level was significantly negatively correlated with fetus development. Low birth weight (less than 2,500 g) occurred significantly more frequently in infants with higher cord blood Cd than in those exposed to lower levels of cord blood Cd (Tein, et al., 2009).