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The metabolism of copper during pregnancy — a review

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Adequate copper supplies are essential for normal foetal development. During pregnancy, many changes occur in copper levels and transport in both mother and foetus. Copper levels in maternal serum rise, more or less in parallel with increases in serum ceruloplasmin. At the same time, total body copper levels increase, but not in the tissues normally associated with copper homeostasis. The placental transport system changes during development, increasing during the latter stages and resulting in the transport of more copper towards the end of gestation than earlier in pregnancy. Most of the copper transferred across the placenta is found in the foetal liver and during pregnancy serum levels in the foetus actually drop. This review examines these changes and relates the observations to changes in copper metabolism in the developing foetus.

INTRODUCTION

During pregnancy, the developing foetus is entirely dependent on the mother for the supply of copper. Adequate supplies are essential for normal foetal development. For example, babies with Menkes' disease, a disorder of copper transport, are born with a wide variety of problems, ranging from neurological disorders to errors in connective tissue structure. Afflicted children usually die within the first 2 years of life (Danks, 1988). During pregnancy there are considerable changes in copper metabolism in both the mother and the developing foetus. Although this has been recognised for many years, the mechanisms underlying the changes are not very well understood. In this review, I will attempt to bring together observations made under a variety of conditions in both humans and animals and will try to correlate the mechanisms of copper metabolism with the observations made at the whole organ or whole animal level. An overview of the processes is given in Table 1.

COPPER METABOLISM IN THE MOTHER

Serum changes in copper and ceruloplasmin

During pregnancy, levels of both copper and ceruloplasmin in maternal serum rise markedly (Fig. 1). The first observations (reported in Linder (1991)) suggested that the copper levels rose from 1·2 μg/ml (approx. 19 μM) to 2·7 μg/ml (42·5 μM) during the first trimester. However it is now generally accepted that the increase is somewhat less, from about 18 μM to 26 μM at the end of the first trimester, finally increasing to about 35 μM (Kiiholma *et al.*, 1984; Ozgunes *et al.*, 1987) by the end of pregnancy. In the rat, Sato and Henkin (1973) found an increase in maternal serum copper towards the end of pregnancy. In the mouse, however, the changes are not so apparent, with serum copper rising from 0·97 μg/ml (approx. 19 μM) to 1·6 μg/ml (25 μM) at day 10 (the gestation period in this strain of mice is about 22 days) thereafter changing little until birth (Erlich & McArdle, unpublished observations).

At the same time, ceruloplasmin levels in maternal serum rise, correlated to an increase in ceruloplasmin mRNA levels in the maternal liver (Thomas *et al.*, 1989). Few studies have examined the ratio of copper to ceruloplasmin. We have preliminary data to suggest that the non-ceruloplasmin copper in the mother's serum increases at a rate greater than that of the ceruloplasmin copper (Tong *et al.*, submitted) which we suggest occurs as a result of increased transport of copper across the maternal gut during gestation.

At the moment, there are no data on how rates of production of ceruloplasmin are regulated. Various workers have shown that oestrogens and progesterone both increase ceruloplasmin production and secretion and it is feasible that the increase occurs as a result of this stimulation. It should also be borne in mind, however, that ceruloplasmin is an acute phase protein and the concentration of other acute phase proteins also rise in serum during pregnancy.

Table 1. Summary of changes which occur in the copper status of the mother and baby during pregnancy

Tissue	Change
Mother	
Liver	—
Bile secretion	↓
Serum copper	↑
Serum ceruloplasmin	↑
Serum Cu/ceruloplasmin ratio	↑
Milk	↑
Bone	—
Foetus	
Liver	↑
Serum	↓
Brain	↑

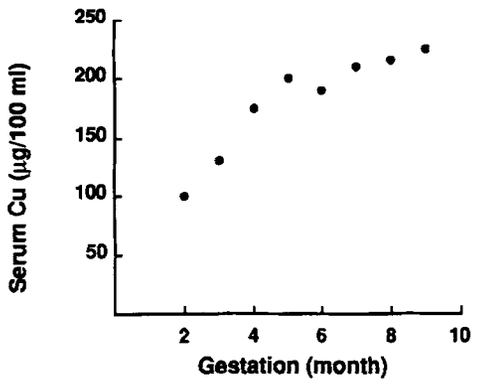

Fig. 1. Copper levels in maternal serum during pregnancy (redrawn from data compiled in Linder (1991)).

Changes in copper levels in other maternal tissues

In parallel with increases in serum there are changes in total maternal copper (excluding the products of conception) (Williams *et al.*, 1977). These workers showed in rats that the total maternal copper (apart from the conceptus) increased to about 10% over controls by mid-gestation and thereafter very rapidly to 50% higher by the end of gestation. Thereafter, levels fell rapidly back to control values.

It is interesting that the measured increase was far greater than alterations in either liver or bone copper, which did not alter to any significant extent. Linder's comment (Linder, 1991) "One wonders where the rest of the accumulation has occurred" is extremely apposite. She postulates that at least some of the copper may be stored in the mammary gland for release in milk after birth, but acknowledged that milk copper concentrations are very variable between species and do not relate well to measured increases in maternal copper.

A second, and critically important, effect of pregnancy is an increase in retention of dietary copper by the pregnant female (King & Wright, 1985). This probably arises as a consequence of decreased biliary excretion (Terao & Owen, 1977) and results, in the rat, in an increase in total body copper content of about 10%, excluding the products of conception, by about midway through pregnancy and rising to about 50% greater by term (Williams *et al.*, 1977). Surprisingly, very little work has been carried out on the effects of pregnancy on copper absorption. Such evidence as there is, and it is very limited, indicates that there is an increase in the transport across the rat gut (see Linder (1991) and discussion above) but how it is regulated or how it changes during gestation is not known.

COPPER METABOLISM IN THE DEVELOPING FOETUS

Changes in copper-dependent enzymes during pregnancy

Copper is central to the function of many enzymes, ranging from cytochromes involved in electron transport, to proteins central to collagen and elastin cross-linking, to proteins involved in catecholamine function. However, most studies have been carried out on CuZn superoxide dismutase (SOD), which is a cytosolic enzyme centrally involved in detoxification of free radicals. In most species, such as guinea pig or rat, levels in the lung are low until birth. After birth, levels of both mRNA and protein SOD rise. The signal for the rise appears to be air breathing, since levels in premature pups are the same as those in animals born at normal term (Bingle *et al.*, 1990). Humans, however, display a different pattern of expression of the protein. In the liver, CuZn SOD increases through gestation as it does in other species but in the lung, expression is already at term levels at 30 weeks gestation (Strange *et al.*, 1990; McElroy *et al.*, 1992). This is a surprising observation, since the protein is unlikely to have a functional role until birth, when the lung starts to breathe air, but it may be related to the fact that the human lung is more differentiated at term than many other mammalian species.

Changes in foetal copper levels

During gestation in all species studied, total copper levels in the foetus rise. The pattern of accretion is very different depending on the species and the tissue studied. However, it is generally true to say that most of the copper is found in the foetal liver, associated with metallothionein, a low-molecular-weight protein rich in cysteine residues (Widdowson *et al.*, 1974; Bremner *et al.*, 1977; Hurley *et al.*, 1980; Andrews *et al.*, 1987). Within the liver, the copper is mostly intranuclear but moves from there into the cytoplasm in the neonatal period (Nartey *et al.*, 1977). The physiological significance of the location is unknown.

In the foetal serum copper levels do not follow the same pattern as in the liver. In humans, concentrations actually fall slightly from the second trimester onwards. In contrast, this is the period when ceruloplasmin levels start to rise, so that the ratio of ceruloplasmin to non-ceruloplasmin copper rises markedly. When the ratio of maternal serum copper to foetal serum copper is

measured, it changes markedly during gestation. With the rise in maternal copper and the fall in foetal serum copper, the ratio nearly doubles (Tong *et al.*, submitted). Against this must be borne in mind the considerable increase in total body copper and, in the rat, observations that suggest that pattern is much more exaggerated (Sato & Henkin, 1973).

The clearest change in foetal copper is in the liver. Copper levels, whether expressed in terms of the whole liver or in terms of wet weight can increase several fold. Depending on the species, the increase can be matched by an increase in zinc levels, but in deer, for example, only copper levels rise (Reid *et al.*, 1980). Why the increase occurs is not clear, however. It is possible that the copper is used for cupro-protein synthesis in the neonatal period but it is clear that the copper stored in the liver is not adequate for normal development and the newborn animal must obtain copper from the maternal milk. Animals fostered to toxic milk mouse dams, which have abnormally low copper levels in the milk, die of copper deficiency (Rauch, 1983). A second possibility is that the liver synthesises metallothionein to act as a store for cysteine during the neonatal period (Andrews *et al.*, 1987) and a third possibility is that the copper is held in the liver only because the excretion mechanism, through the bile, is not patent during intrauterine life.

We argued that if there was a specific transfer system for copper from mother to foetus, and that the system changed in activity or characteristics during pregnancy, then the latter possibility could be discounted and hence we examined the transfer of copper from mother to foetus at different stages of gestation in the mouse (McArdle & Erlich, 1991). Our data and that of others approaching the same problem is summarised in the following sections.

DEVELOPMENT OF THE PLACENTAL COPPER TRANSPORT SYSTEM DURING PREGNANCY — *IN VIVO* STUDIES

The first systematic attempt to study copper transfer from mother to foetus were undertaken by Romeu *et al.* (1986) in the rat. They showed that the rate of transfer of copper increased during pregnancy, reaching a maximum at day 16, thereafter remaining constant until term. A similar pattern is seen in the mouse. The distribution of copper and the rates of transfer were measured by injecting $^{67}CuHis_2$ intravenously. At different times following injection, the mice were killed and tissue samples taken, counted and also analysed for total copper. The data showed that transfer was maximal at day 16, thereafter dropping again towards term. The rate increased whether expressed as an amount transferred per foetus or per g foetal weight, showing that there was a rise over and above that which could be accounted for by a simple increase in foetal size (McArdle & Erlich, 1991).

However, when the different parts of the foetus are examined separately, a slightly different pattern is seen (Fig. 2). The foetal liver accounts for more than 90% of the copper transferred and the rate of accumulation matches that shown in the whole animal. In the rest of the foetus, that is without the liver being included, levels remain constant until day 16 or 17 then drop, as also is seen in the foetal serum of humans (Fig. 2(b)). This data was extended by Linder's group (Lee *et al.*, 1993), who investigated the fate of ^{67}Cu injected as ^{67}Cu-albumin, or ^{67}Cu-ceruloplasmin into pregnant rats. They found that either substrate could deliver copper to the foetus and that the copper in the foetal circulation was associated with albumin and a protein only really described by Linder's group called transcuprein (Wirth & Linder, 1985).

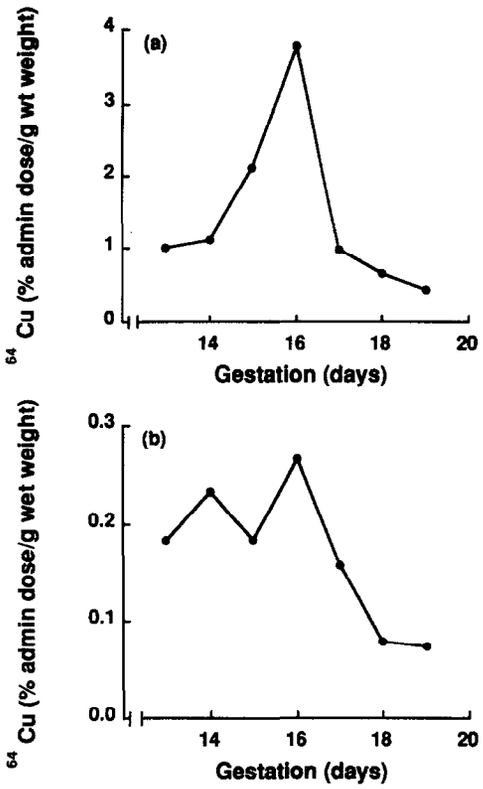

Fig. 2. (a) Transfer of copper from maternal serum to foetal liver. Animals were injected with ^{64}Cu as $CuHis_2$ and killed 1 h later. The data are expressed as % of the injected dose since we cannot determine the specific activity of the serum copper. These data are taken from McArdle and Erlich (1991) and from Erlich and McArdle (unpublished data). (b) Transfer of copper from maternal serum to the remainder of the foetus. Animals were treated as described in (a) and data expressed in the same way. Data are taken from Erlich and McArdle (unpublished work).

These papers suggest firstly that there is a transport mechanism capable of removing copper from a maternal carrier, either histidine, albumin or ceruloplasmin, and transferring the copper across the placenta to the foetus and mainly to the foetal liver. The data further show that the transport system changes during pregnancy, increasing the rate of transfer either by increasing transporter number or affinity, probably the former, and that the pattern of change is different in different species. The remainder of this review,

therefore, will concentrate on this transport process, examining our knowledge of the processes involved in the movement of copper from mother to foetus across the placenta. Surprisingly, little work has been carried out in this important area, but based on these studies and work carried out in other cells some conclusions can be drawn.

THE ROLE OF CERULOPLASMIN IN COPPER METABOLISM

As discussed above, the developing foetus must obtain its copper from maternal serum. There are two main forms of copper in serum; ceruloplasmin, which carries about 80–90% total copper, and albumin and amino-acid-bound copper, which comprises the remainder. One of these two pools must provide the copper from the foetus.

Ceruloplasmin has been suggested as the copper transport protein for many years (Marceau & Aspin, 1972, 1973) and ceruloplasmin receptors have been identified on many different cell types, varying from endothelial cells of the liver (Tavassoli, 1985; Tavassoli *et al.*, 1986) and aorta (Stevens *et al.*, 1984), red blood cells (Barnes & Frieden, 1984) to differentiating K562 cells (Percival & Harris, 1988, 1990). Copper from ceruloplasmin has been shown to be incorporated into SOD (Dameron & Harris, 1987*a,b*) but these workers also showed that copper from albumin or indeed as ionic copper could also be incorporated into the protein.

Similarly, Barnea and co-workers have identified a $CuHis_2$ transport mechanism in brain (Barnea *et al.*, 1988; Harrter & Barnea, 1988), and Herd *et al.* (1987) have demonstrated a similar system in other cells. Apart from being the major copper protein in serum, ceruloplasmin is also a ferroxidase and has been proposed to have a role in iron metabolism in the liver itself (Frieden, 1980). It is an acute phase protein and, in fact, it has been suggested that it rises during pregnancy because it is an acute phase protein and not because of any function in copper metabolism.

PLACENTAL COPPER TRANSPORT — *IN VITRO* STUDIES

Placental cells can be isolated and cultured from term placenta. The cells are predominantly cytotrophoblast but will, given time, differentiate to syncytiotrophoblast cells. Mas and Sarkar (1992) were the first to use this system to study copper uptake. They investigated whether the form of copper presented to the cell made any difference to the initial rate of uptake and found that copper from Cu-albumin was taken up less readily than any other form, including $CuCl_2$! Over an incubation period of 60 min, histidine stimulated uptake, suggesting that the cells preferred $CuHis_2$ as a substrate to $CuCl_2$. The kinetics of uptake were very dissimilar to uptake of histidine alone, as has been shown for hepatocytes (McArdle *et al.*, 1988), indicating that $CuHis_2$ is not taken up by the histidine transport system. One other observation these workers made was incubating the cells with unlabelled ceruloplasmin for 2 h resulted in a decrease in both ceruloplasmin activity and copper content of the medium, indicating that the placental cells had taken up and/or broken down the protein. These workers deduced that placental trophoblast cells accumulated copper preferentially from ceruloplasmin, although other complexes could also form the substrate for uptake, and once the metal was taken up it was handled by the cells in the same way, irrespective of its initial derivation.

We have followed up this work and have characterised the uptake process in more detail. In trophoblast cells in culture, uptake is via a carrier-mediated process (Tong & McArdle, abstract submitted), is independent of cellular energy or cellular ion gradients, and the k_m for uptake by whole cells is similar to that already described for placental vesicles ((McArdle & Van den Berg, 1991) and see below).

At the maternal face of the placenta, the cells form a syncitium and the apical membrane is folded into many microvilli. These can be isolated in high yield and form sealed vesicles which can be used to study uptake across the microvillar membrane. Using $CuHis_2$ as the substrate for copper uptake, we identified a carrier-mediated transport system, which showed temperature and concentration dependence (McArdle & Van den Berg, 1991). The kinetics of uptake were similar to those described for other cell types, with k_m values in the range appropriate for the low-molecular-weight copper pool (Schmitt *et al.*, 1983; McArdle *et al.*, 1988).

Linder and colleagues were the first to suggest that the membrane carrier for copper could recognise the metal in a variety of different complexes (Orena *et al.*, 1986). They showed that CuNTA (nitrilotriacetate) could block copper uptake from ceruloplasmin by Chinese hamster ovary (CHO) cells. This work was extended by Harris and co-workers (Dameron & Harris, 1987*a,b*), who showed that any copper complex presented to aortic endothelial cells could reactivate superoxide dismutase. Our preliminary data (McArdle & Van den Berg, 1991) supported these observations in the placenta, where we showed that ceruloplasmin inhibited uptake of copper from $CuHis_2$ complexes.

We have now extended this work (Hilton *et al.*, submitted) and have, as a consequence, provided some new information on the way the placenta transports copper. Ceruloplasmin interferes with copper uptake by the placenta in a complex manner. The kinetics of copper uptake by vesicles isolated from human placenta show a degree of co-operativity which we have interpreted as one molecule binding which in turn facilitates the uptake of the second. When ceruloplasmin competes for uptake, the co-operativity disappears and both the affinity and the maximum rate of uptake are altered. These data can be interpreted most simply by assuming that there are two binding sites on the

transporter. Binding of ceruloplasmin can occur to either site and when it binds it blocks binding of $CuHis_2$ to that site, but not to the other. This will seem to reduce the co-operativity and would also decrease the apparent affinity and V_{max}. We have previously suggested this model may be likely (McArdle & Van den Berg, 1992).

Is this a likely hypothesis and what relevance does it have to copper metabolism in the pregnant mammal? The answer to the first question is clearly "yes it is likely." In patients with Wilson's disease, ceruloplasmin levels are low, yet copper levels in non-hepatic tissues, especially in brain, are very high. Similarly, in those few patients with aceruloplasminemia, copper levels are normal (see Danks (1988)). Clearly, then, forms of copper other than ceruloplasmin can provide the tissues with their copper requirement. In vivo studies showing the essential involvement of ceruloplasmin in copper delivery cannot readily be performed, but Linder and co-workers (Lee *et al.*, 1993) have studied the kinetics of copper transport across the rat placenta carefully and have shown that ceruloplasmin copper is preferentially delivered to the foetus. Our own data would support this observation, since we found that ceruloplasmin can inhibit copper uptake by placenta by 50% at much lower concentrations than that of $CuHis_2$; in other words, the transport system prefers ceruloplasmin (Hilton *et al.*, submitted).

Once inside the placenta, the mechanism for transfer is a mystery. Glutathione may be involved in the transfer, since drugs which inhibit glutathione synthesis also decrease copper uptake by cultured placental cells (Tong & McArdle, abstract submitted). Foetuses with Menkes' disease are born copper deficient, indicating that the disorder is expressed in the placenta. The defective protein has been shown to be a transporting ATPase (Chelly *et al.*, 1993; Mercer *et al.*, 1993; Vulpe *et al.*, 1993) which is located somewhere within the placental barrier. Where it is located remains, at the moment, unknown.

In summary, we are beginning to understand how copper is transported from mother to foetus during pregnancy. Uptake into the placenta is normally from ceruloplasmin but can be from other copper complexes and occurs via a carrier mediated system. At some stage in the transfer, a pump may be involved, possibly at the basal membrane, but perhaps also in some intracellular membrane system. The copper may be moved as Cu^{2+} or, more likely, as Cu^{+}.

The question arises as to whether these studies have more than a scientific value. Clearly, when estimating if a mother is copper deficient, it is essential to measure the appropriate form of copper. Similarly, we know that babies born prematurely are low in liver copper and it is critically important to understand whether this represents a risk to their future well-being. Finally, there are many conditions where placental size and efficiency of transfer change. It is necessary to understand if this is likely to lead to a fall in the delivery of copper from the mother to the baby. Only with an understanding of the processes involved will we be able to monitor foetal development properly and to design strategies for intervention and assistance when necessary.

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