ELSEVIER

Biochimica et Biophysica Acta 1291 (1996) 53–59

BBA Biochimica et Biophysica Acta

The effects of iron loading and iron deficiency on the tissue uptake of ^{64}Cu during development in the rat

Andrew Crowe, Evan H. Morgan *

Department of Physiology, University of Western Australia, Nedlands, Perth, Australia 6907

Received 22 March 1996; revised 22 April 1996; accepted 22 April 1996

Abstract

This study examined the uptake of ^{64}Cu by the brain, liver and other organs during development in rats aged 15, 21 and 63 days fed low, normal and high iron diets, using either a solution of $^{64}CuCl_2$ chelated with nitrilo-triacetic acid (NTA) or ^{64}Cu-ceruloplasmin (^{64}Cu-Cp). ^{64}Cu-NTA uptake was higher in the brain, spleen, kidneys, femurs and red cells at 15 days than at the later ages, while the liver took up most of the ^{64}Cu in 63-day-old rats over the 2 h of the study. The brain had similar levels of ^{64}Cu-NTA uptake at 15 and 21 days, even though liver uptake significantly increased, suggesting that Cu-NTA uptake by the brain increases from 15 to 21 days. The brain took up a greater percent of the injected dose of ^{64}Cu-Cp than ^{64}Cu-NTA yet, in either case, brain uptake was lower than that of the other organs. Iron loaded rats had significantly higher uptake of non-ceruloplasmin-bound ^{64}Cu in all the organs examined, for at least one of the three ages, when compared with control rats. However, iron deficiency produced little change. Iron loading has a greater effect on ^{64}Cu-Cp uptake than ^{64}Cu-NTA, decreasing ^{64}Cu uptake in the brain, liver, kidneys and femurs. Iron deficiency only increased ^{64}Cu-Cp uptake in the liver. These results suggest that the mechanism of copper uptake by the liver is still maturing during suckling in the rat, and that ceruloplasmin receptor numbers are down regulated by iron loading, thus providing evidence of a new link between iron and copper metabolism.

Keywords: Iron loading; Iron deficiency; Fe; Copper; ^{64}Cu-uptake; Neonatal tissue development; (Rat)

1. Introduction

Copper and iron are both essential for normal cellular metabolism, functioning in such capacities as haem synthesis and electron transport [1–3]. Both of these metals are highly reactive, which explains their role in many enzymic processes. However, this reactivity can also be associated with damage to cellular systems if the ions are free in the cell or extracellular fluid. Extracellulary this danger is, to a large extent, overcome by complexation to proteins. Iron is carried in the plasma by an 80 kDa protein, transferrin, while copper utilises other carriers, such as albumin, transcuprein, amino acids and a 132–134 kDa protein, ceruloplasmin, which transports over 75% of plasma copper [4–7]. Most organs, including the brain [8] are thought to aquire copper through at least two different processes, using free copper and ceruloplasmin mediated pathways [9,10]. Ceruloplasmin also functions as a serum antioxidant, is involved in endogenous modulation of inflammatory responses and has ferroxidase activity [11,12].

Many studies have examined the interactions between different dietary levels of iron and copper on the tissue concentrations of each metal. Reports of increased blood, plasma, liver and kidney copper concentrations in iron deficiency are common [1,13]. Iron loading also has an effect, decreasing copper levels in the liver, but in some cases, the animals must already be copper deficient for the effect of increased iron intake to become significant [14,15]. However, the mechanisms of copper uptake in relation to its interaction with iron are not fully understood, and we are not aware of any studies examing such interactions during postnatal development. Investigation of the interactions between the uptake of iron and copper by the brain in young developing animals are particularly important due to the susceptibility of the developing brain to toxicological damage [16–19], and the recent observations of increased deposition of iron in the brain in humans with aceruloplasminaemia [20–22]. Liver copper levels show large changes during the course of early postnatal development [23,24],

* Corresponding author. Fax: +9 380 1025; e-mail: ⟨ehmorgan@uniwa.uwa.edu.au⟩.

PII S0304-4165(96)00044-X

yet the activity of the mechanisms involved in copper uptake during postnatal development has recieved little attention.

In this study we have examined the effect of dietary iron loading and iron deficiency on the uptake of copper from the plasma by the brain, liver and other organs during postnatal development in the rat. Intravenously injected ^{64}Cu-NTA and ceruloplasmin-bound ^{64}Cu (^{64}Cu-Cp) were used to determine copper uptake by the organs. It is important to understand the relationships between iron and copper uptake into the tissues of the body, especially with all the new evidence linking the metabolism of these two metals in human disorders [20–22].

2. Materials and methods

2.1. Materials

Copper-64 ($^{64}CuCl_2$, 220–260 MBq/mg) was purchased from Australian Radioisotopes Industries (Lucas Heights, Sydney, Australia). Carbonyl iron was purchased from ISP (Australasia), Silverwater, NSW. Nitrilo-triacetic acid (NTA) was from BDH-AnalaR, Poole, England. Methoxyflurane (Penthrane) was from Abbott Hospital Products, Kurnell, NSW, Australia. Sodium pentobarbitone was from Boehringer Ingelheim, Artarmon, NSW, Australia and all other chemicals were from Sigma, St. Louis, MO, USA.

2.2. Animals

The experiments were performed with rats of a Wistar strain. Iron deficiency was produced as described earlier [25], by feeding a low-iron semi-purified diet, commencing at day 18 to 19 of pregnancy as determined by the observation of spermatozoa in the vagina. The iron content of this diet was 5–10 mg/kg. Controls for the iron deficient rats received tap water and the same diet to which was added ferrous ammonium sulphate (1.3 g/kg). This diet was classified as iron sufficient. Iron loading was modified from a method previously described in our laboratory [25] by feeding rats with 2% carbonyl iron mixed with a crushed version of standard rat chow which contained 170 mg of Fe/kg (Glen Forrest Stockfeeders, Glen Forrest, Western Australia) and tap water. Controls for the iron loaded rats were given the standard rat chow and tap water. These diets were given to mothers at day 18 to 19 of pregnancy and were continued with the dams and young rats until the age of 15, 21 and 63 days.

2.3. Preparation of ^{64}Cu-ceruloplasmin.

Radiolabelled ceruloplasmin was prepared using the method described by Lee et al. [26]. Briefly, $^{64}CuCl_2$ in 0.1 M HCl was chelated with NTA at a 1:1 molar ratio. The solution was neutralised with 1.0 M NaOH and immediately injected intravenously into an anaesthetised rat weighing 150 g. 24 h after the injection of the labelled Cu-NTA almost all of the ^{64}Cu present in the plasma should be bound to ceruloplasmin [26]. Hence, after 24 h, the injected rat was sacrificed with an intraperitoneal injection of sodium pentobarbitone. The blood was collected from the right ventricle and centrifuged at $1000 \times g$ for 10 min. The plasma was separated and used as the ^{64}Cu-Cp injection solution. Samples of plasma was fractionated on a Sephacryl S-200 column. This confirmed that the ^{64}Cu was protein bound.

2.4. Experimental procedure

Rats aged 15, 21 and 63 days were used to study the tissue uptake of ^{64}Cu-NTA, and aged 21 and 63 days for the uptake of ^{64}Cu-Cp. Each rat was injected through a lateral tail vein with a solution of $^{64}CuCl_2$ prepared as described above (approx. 3.0 MBq per rat) or ^{64}Cu-Cp in 0.15M NaCl (approx. 0.3 MBq per rat), using a glass syringe and a 30 gauge dental needle. Rats were under methoxyflurane anaesthethia during this procedure. 15- and 21-day-old rats were injected with 100 μl of solution while 63-day-old animals were injected with 250 μl. At 5 min and 30 min after the initial injection, approx. 50 μl of blood was collected in a heparinised microhaematocrit tube through an incision of the ventral tail vein. After centrifugation, the haematocrit was recorded and an aliquot of the plasma counted for radioactivity.

2 h after the isotope injection the animals were anaesthetised with an intraperitoneal injection of sodium pentobarbitone. The abdomen and chest were opened and blood obtained from the right ventricle with a heparinised syringe using a 23 or 25 gauge needle. The right ventricle was incised and the animal perfused through the left ventricle with heparinised 0.15 M NaCl at 4°C using a 20 ml syringe with a 23 gauge needle. The volume of perfusate varied between 20 and 70 ml depending on the animal's size. The brain, liver, kidneys, spleen and femurs were removed from all rats.

Radioactivity was measured in the plasma samples, the organs and aliquots of blood cells after washing 3 times in ice-cold 0.15 M NaCl. All radioactivity measurements were corrected for the short half life of ^{64}Cu.

2.5. Analytical methods

Liver non-haem iron was determined as described by Kaldor [27]. Radioactivity was counted in a γ-scintillation counter (LKB-Wallace 1282 Compu-gamma). Statistical analysis of the results was performed by analysis of variance (ANOVA). When differences were detected ($P \leq 0.05$), means were tested with Fisher's protected least significant difference (PLSD) post hoc test [28]. Evidence

of statistically significant differences were considered to be P values of less than 0.05.

3. Results

The presence of iron deficiency in rats fed the low-iron diet was demonstrated by significant reductions in packed cell volume (PCV) and decreased liver NHFe in the 21- and 63-day-old rats (Table 1). Iron overload was also demonstrated by increased liver NHFe in the 15-, 21- and 63-day-old rats (Table 1).

3.1. Copper-NTA

Plasma clearance of ^{64}Cu was rapid in all of the animals, regardless of iron status, 30–35% being cleared within 3 min of injection, and after 2 h almost no ^{64}Cu could be detected (Fig. 1). Clearance was more rapid with increasing age. Altered iron status had no effect on the plasma clearance rates of ^{64}Cu-NTA. Therefore, for clarity, only the results from control rats are shown (Fig. 1).

In control and iron loaded rats, the uptake of ^{64}Cu from NTA per gram of tissue decreased in all the organs examined as the rats aged from 21 to 63 days (Fig. 2). In the liver of control and iron loaded rats there was a rise between 15 and 21 days and then a fall to 63 days. Uptake by the whole liver increased to reach 75 to 95% of the injected dose in the 63-day-old rats compared to only 10% at 15 days. Uptake by the whole brain remained relatively constant during the suckling period, and then fell to a very low level by 63 days, while total uptake by the kidneys and spleen was unaffected by age and that of the femurs decreased markedly between 15 and 21 days, and further

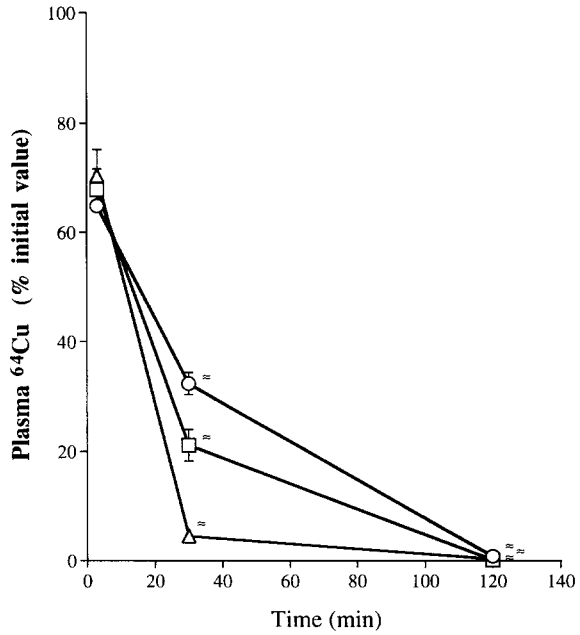

Fig. 1. ^{64}Cu remaining in the plasma of control rats at 3, 30 and 120 min after intravenous injection of ^{64}Cu-NTA in 15- (○), 21- (□) and 63- (△)) day-old rats. Results are expressed in % dose in total plasma. Results for iron loaded, iron sufficient and iron deficient rats were similar to those of controls. Significant difference in relation to age increase = ≈ ($P < 0.05$).

by 63 days. Iron loading resulted in significantly greater total uptake of ^{64}Cu by the brain, femurs and red cells at 15 days of age, the kidney at 21 days and the liver at 63 days; only in the spleen at 21 days was there any evidence of decreased uptake (Fig. 2). The brain showed the lowest uptake of ^{64}Cu of all the organs examined, only 0.1% of injected dose at 15 days, and about 0.01% at 63 days.

The uptake of ^{64}Cu by the organs of iron sufficient and iron deficient rats showed the same pattern of changes as the animals aged from 15 and 63 days as was observed for rats fed the control and iron loaded diets (Fig. 3). Iron deficiency had little effect on ^{64}Cu uptake. It resulted in significantly higher ^{64}Cu uptake at 15 days in the brain, 21 days in the liver and both 15 and 63 days in the spleen; and the kidney had decreased ^{64}Cu uptake at 63 days.

Uptake of ^{64}Cu into red blood cells decreased rapidly from 15 to 21 days, then at a slower rate from 21 to 63 days (Figs. 2 and 3). Iron loaded rats had a higher ^{64}Cu uptake into red blood cells than controls at 15 days only (Fig. 2), while iron deficiency had no significant effect compared to iron sufficient rats (Fig. 3).

3.2. Copper-ceruloplasmin

Plasma clearance of ^{64}Cu from ceruloplasmin was much slower than that of ^{64}Cu-NTA in both the 21- and 63-day-old rats. Less than 5% of ^{64}Cu-Cp was cleared within 3 min of injection, and between 5 and 7% of injected dose

Table 1
Packed cell volume and liver non-haem iron in control, iron loaded, iron sufficient and iron deficient rats

Diet type	Age (days)	PCV (%)	Liver NHFe (μg)
Con	15	29.0±0.7	7.5±0.3
	21	33.7±1.5 ≈	131±17 ≈
	63	42.1±1.0 ≈	805±41 ≈
Fe^{2+}	15	35.0±1.3 *	342±30 *
	21	37.1±1.8	2040±193 * ≈
	63	42.9±0.5 ≈	19053±1394 ≈
$Fe^{\pm}$	15	31.4±0.7	9.5±1.0
	21	34.9±0.9 ≈	188±56 ≈
	63	40.3±0.7 ≈	1439±95 ≈
Fe^{2-}	15	29.6±0.7	8.3±0.5
	21	24.0±0.4 # ≈	18.9±2.1 # ≈
	63	30.0±3.0 # ≈	66.0±4.5 # ≈

Con, rats fed a control diet; Fe^{2+}, rats with carbonyl iron added to a control diet; Fe^{2-}, rats fed an iron deficient diet; $Fe^{\pm}$, rats fed an iron deficient diet with added iron equivalent to control iron concentrations. Significant difference between Con and Fe^{2+} rats at the same age + * ($P < 0.05$); significant difference between $Fe^{\pm}$ and Fe^{2-} rats = #($P < 0.05$); Significant difference in relation to age increase = ≈ ($P < 0.05$).

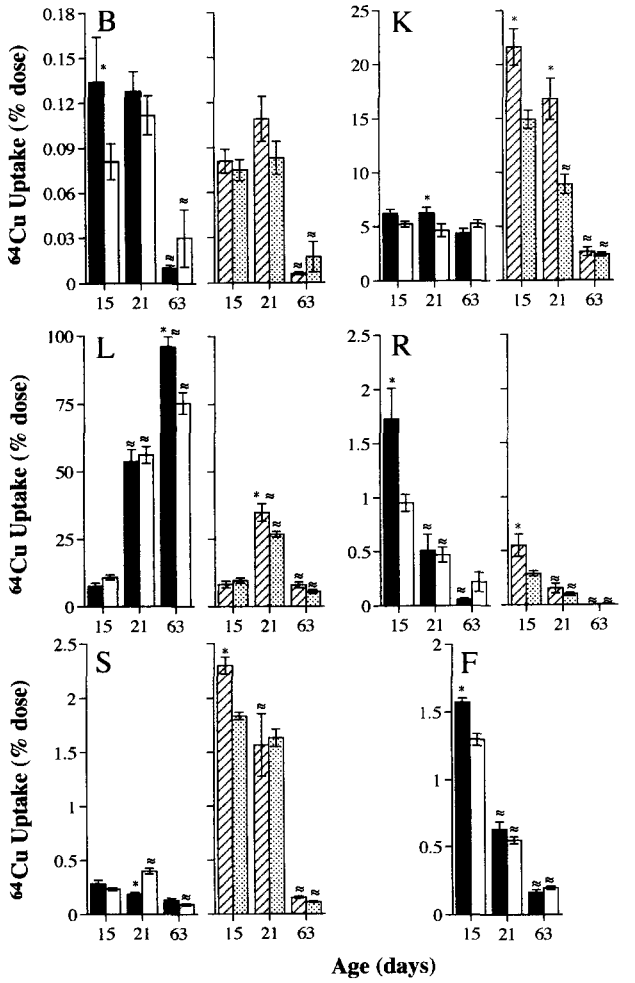

Fig. 2. Uptake of ^{64}Cu in the brain (B), kidneys (K), liver (L), red cells (R), spleen (S) and femurs (F) of control (□, dotted column) and iron loaded (■, striped column) rats 2 h after intravenous injection of ^{64}Cu-NTA in 15-, 21- and 63-day-old rats. Results are expressed as % dose per whole organ (■,□) and % dose per g organ weight (striped column, dotted column), or in the case of red cells, % dose per ml blood. Each value represents the mean ± S.E. of 4 or 5 rats. Significant difference between control and iron loaded rats at the same age = * ($P < 0.05$); Significant difference in relation to age increase = ≈ ($P < 0.05$).

was still remaining in the plasma after 120 min. Animals with altered iron status did not show any significant difference in plasma clearance compared to controls.

The brain took up to 4 times the percentage of the injected dose of ^{64}Cu from ceruloplasmin as it did from Cu-NTA at both ages examined (21 and 63 days), but it still had the lowest uptake of ^{64}Cu of any of the organs examined (Table 2). By contrast, hepatic uptake of ^{64}Cu from ceruloplasmin was much lower than from NTA (Table 2). The uptake of ^{64}Cu from ceruloplasmin by the kidneys was approximately the same as that of the livers when examined as a % dose per gram of tissue. Iron loading was associated with reduced uptake of ^{64}Cu in the brain of 21-day-old rats compared to controls and with reduced uptake of ^{64}Cu in the whole liver of 21- and 63-day-old rats. Kidneys and femurs from iron loaded rats also had reduced uptake of ^{64}Cu compared to controls, but it was not apparent until 63 days in these organs (Table 2).

Previous work from this laboratory has shown that the greatest effect of iron deficiency on iron uptake by the brain, relative to controls, is at 21 days [25,29]. Therefore, this age was chosen preferentially to examine the effects of ^{64}Cu-Cp uptake during iron deficiency. However, the uptake of ^{64}Cu-Cp by the brain was not significantly affected by iron deficiency. In contrast, the liver and spleen took up significantly more ^{64}Cu-Cp during iron deficiency than in controls when examined as % dose per gram of tissue.

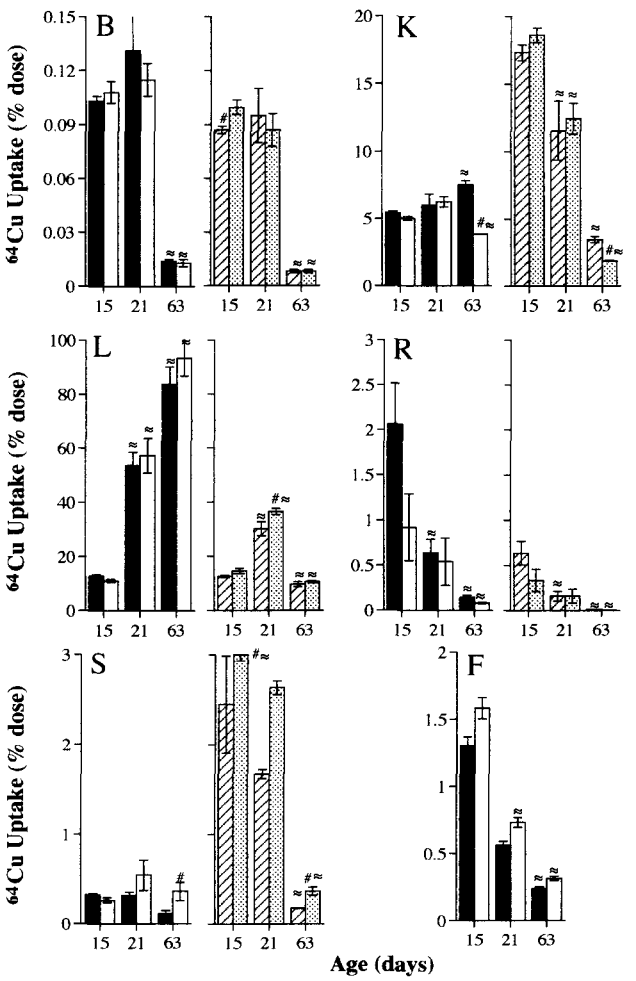

Fig. 3. The uptake of ^{64}Cu in the brain (B), kidneys (K), liver (L), spleen (S) and femurs (F) of iron sufficient (■, striped column) and iron deficient (□, dotted column) rats 2 h after intravenous injection of ^{64}Cu-NTA in 15, 21 and 63 day rats. Results are expressed as % dose per whole organ (■,□) and % dose per g organ weight, or in the case of red cells, % dose per ml blood (striped column, dotted column). Each value represents the mean ± S.E. of 4 or 5 rats. Significant difference between iron sufficient rats and iron deficient rats at the same age = #($P < 0.05$); Significant difference in relation to age increase = ≈ ($P < 0.05$).

Table 2
Organ uptake of ^{64}Cu in control, iron loaded and iron deficient rats 2 h after intravenous injection of ^{64}Cu-ceruloplasmin

Organ type	Age (days)	^{64}Cu uptake					
		% dose			% dose/g		
		control	iron loaded	iron deficient	control	iron loaded	iron deficient
Brain	21	0.31 ± 0.06	0.14 ± 0.04 *	0.20 ± 0.03	0.23 ± 0.04	0.11 ± 0.03 *	0.16 ± 0.03
	63	0.09 ± 0.02	0.07 ± 0.02		0.05 ± 0.01	0.05 ± 0.01	
Liver	21	30.3 ± 8.20	13.4 ± 0.33 *	25.2 ± 1.98	13.8 ± 3.74	7.86 ± 0.057	21.0 ± 1.97 #
	63	28.9 ± 9.78	8.82 ± 0.49 *		2.39 ± 0.87	0.96 ± 0.11 *	
Kidney	21	5.76 ± 1.64	3.82 ± 0.12	3.87 ± 0.35	10.2 ± 3.04	8.34 ± 0.56	9.11 ± 1.05
	63	9.48 ± 3.02	1.85 ± 0.19 *		4.08 ± 1.37	1.15 ± 0.14 *	
Spleen	21	1.01 ± 0.25	0.46 ± 0.06	0.80 ± 0.10	3.62 ± 0.86	2.48 ± 0.37	5.80 ± 0.66#
	63	0.50 ± 0.06	0.45 ± 0.02		0.70 ± 0.08	0.59 ± 0.05	
Femur	21	1.44 ± 0.27	1.18 ± 0.23	1.00 ± 0.0e #			
	63	1.63 ± 0.35	0.57 ± 0.06 *				

Values are the mean ± S.E. of 4 or 5 rats. Significant difference between control and iron loaded rats at the separate ages = * ($P < 0.05$): significant difference between control and iron deficient rats at 21 days = #($P < 0.05$).^{64}Cu-ceruloplasmin was not examined in 15-day-old rats because of the difficulty in obtaining adequate sample volumes.

Only in the femurs was ^{64}Cu-Cp uptake lower in iron deficient rats than in controls (Table 2).

4. Discussion

It has been shown that intravenously injected radiolabelled copper, whether free or complexed with NTA, binds rapidly to albumin and transcuprein [6,30,31]. Hence, it is likely that during the 2 h of this study, much of the ^{64}Cu taken up by the organs after the intravenous injection of ^{64}Cu-NTA was derived from ^{64}Cu-albumin and transcuprein. However, these proteins bind copper reversibly and with relatively low affinity and release copper to the organs so that uptake is similar to that found with free or NTA-chelated copper, as shown by studies using analbuminaemic rats [32]. 2 h is not sufficient time for radiolabelled ceruloplasmin to appear in the circulation [6], so that any uptake shown by organs after the injection of protein free ^{64}Cu was not due to this protein. Incorporation of ^{64}Cu into plasma ceruloplasmin is not observed until about 6h and then rises to reach maximal levels at 24 h [6], the time used to prepare radiolabelled ceruloplasmin in the present work. Also, ^{64}Cu injected as the ceruloplasmin form does not dissociate in the plasma but remains bound to this protein in plasma for over 6 h [26], indicating that the ^{64}Cu taken up by the organs during the 2-h period after injection of ^{64}Cu-Cp must have come from this protein.

The patterns of age-related changes in the uptake of ^{64}Cu by the organs after injecting ^{64}Cu-NTA varied from organ to organ but with each organ were very consistent between the different dietary treatment groups. It is not possible to calculate copper uptake per organ in absolute units, but the data in Figs. 2 and 3 show how the uptake of non-ceruloplasmin-bound copper by the organs, relative to each other, changes with age. These relative changes may be due to age-related alterations in the mechanism of cellular copper transport or to alterations in the availability of injected ^{64}Cu as a consequence of changes in such factors as blood flow or plasma clearance rate. The latter explanation could account for the changes in uptake by kidney, red cells, spleen and femurs, all of which decreased on a per g weight basis as the rats aged and the rate of plasma clearance increased. However, the results for the brain and liver cannot be explained in this way. Brain uptake did not decrease between the ages of 15 and 21 days, although liver uptake increased markedly and, probably as a consequence, plasma clearance of ^{64}Cu increased. Also, between 21 and 63 days the decrease in percentage uptake of ^{64}Cu by the brain was relatively greater than in any other organ except the spleen. These results indicate that the copper uptake capacity of the blood-brain barrier and/or the cerebrospinal fluid barrier increased between the ages of 15 and 231 days and therafter declined. However, the percent of plasm ^{64}Cu transported by these barriers was very low at all ages when compared with the other organ which were examined. Thus, they do act as barriers against copper uptake by the brain and are more effective in this regard in the mature than in the suckling rat.

The age-related changes in the uptake of non-ceruloplasmin-bound ^{64}Cu by the liver are in marked contrast to those of the other organs. Even on a per gram organ weight basis, there was no decline between 15 and 63 days of age, and between 15 and 21 days the uptake increased markedly. Thus, there appears to be an increase in capacity for uptake by liver cells between 15 and 21 days of age and, thereafter a slow decline per cell which was more than matched by the increase in liver cell mass. However, the decline may be more apparent than real since in the 63 day control rats the liver took up approx. 95% of the injected ^{64}Cu in the 2 h study period. Hence, there was

little possibility of increasing the percentage of injected dose which accumulated in the liver, and the uptake capacity of liver cells may not have decreased between 21 and 63 days. The high level of uptake of intravenously injected ^{64}Cu-NTA by the liver of the adult rat has been noted previously [6,10,33], but changes during the suckling period have not been reported. However, Mearick and Mistilis [23] did study suckling rats aged 6 to 28 days, 24 h after injecting ^{64}Cu acetate intraperitoneally. They found evidence of increased excretion into the gut with age. Hence, the rapid clearance of ^{64}Cu-NTA from the plasma and the low level of ^{64}Cu uptake by the liver found in the present work cannot be explained by a more rapid excretion of the radioisotope from the liver at 15 days than at older ages.

The age-related changes in the organ uptake of ceruloplasmin-bound ^{64}Cu were quite different from those of ^{64}Cu-NTA. Since the uptake of Cu-Cp by many different types of cells in vitro has been reported to be dependent on the binding of ceruloplasmin to receptors on the cell-membrane [9,34–37], the uptake found in vivo in the present work probably reflects the relative numbers of these receptors in the different organs and the changes which occur with age. The rate of clearance of ^{64}Cu-Cp from the circulation was slower than that of ^{64}Cu-NTA and the percentage uptake of ^{64}Cu-Cp by the brain at 21 and 63 days and the kidneys, spleen and femurs at 63 days was greater than that of ^{64}Cu-NTA, while hepatic uptake of ^{64}Cu-Cp was much lower than that of ^{64}Cu-NTA at 63 days. Overall, these results imply that as the liver matures during the suckling period of the rat, it takes up an increasing proportion of the non-ceruloplasmin-bound copper in the plasma (such as that derived from intestinal absorption), excretes some in the bile [10,23,33,37] and secretes another portion in the form of ceruloplasmin [6], which is probably the major source of copper for the brain where it can cross the blood-brain barrier by a specific transport mechanism which is more active at the age of 21 days than in the mature rat. Together with recent results from our laboratory showing brain copper concentrations to be highest at 21 days of age [38], it may be concluded that in the rat, the brain receives a large proportion of its complement of copper during suckling and then down regulates the process of uptake. This is very similar to the situation with regard to iron uptake [39] where it has been shown that the decreased rate of uptake with age is correlated with and probably due to decreased numbers of transferrin receptors on brain capillary endothelial cells [25,39].

Iron loaded rats showed some significant increases in the uptake of non-ceruloplasmin-bound ^{64}Cu when compared with control rats. However, iron deficiency produced little change. Therefore it is unlikely that the changes in copper uptake are due to alterations in any transport system which is regulated by iron status. Instead iron loading may stimulate a copper uptake pathway, either as a physiological response or due to iron-induced alterations in cell membrane structure and function which are not specific for copper transport.

Iron loading produced relatively greater changes in the uptake by the brain, liver, kidney and femurs of ^{64}Cu-Cp than of ceruloplasmin-free ^{64}Cu, and the direction of change was a decrease, rather than an increase. Since the uptake of ceruloplasmin-bound copper by most organs is probably dependent on the function of cell membrane receptors, these results indicate that receptor numbers may be down regulated by iron loading and provides evidence of a possible new link between iron and copper metabolism. The opposite type of change, an increase in uptake of ^{64}Cu-Cp in iron deficiency was found only with the liver and spleen, but it may be part of the same mechanism of interaction between the two metals.

References

[1] Davis, G. and Mertz, W. (1987) in Copper in Trace Elements in Human and Animal Nutrition (Mertz, W., ed.), pp. 301–364, Academic Press, San Diego.
[2] Lash, A. and Saleem, A. (1995) Ann. Clin. Lab. Sci. 25, 20–30.
[3] Aisen, P. (1994) in Iron Metabolism in Health and Disease (Brock, J., Halliday, J., Pippard, M. and Powell, L., eds.), pp. 1–30, W.B. Saunders, London.
[4] Hsich, H. and Frieden, E. (1975) Biochem. Biophys. Res. Commun. 67, 1326–1331.
[5] Noyer, M., Dwulet, F., Hao, Y. and Putnum, F. (1980) Anal. Biochem. 102, 450–458.
[6] Weiss, K. and Linder, M. (1985) Am. J. Physiol. 249, E77–E88.
[7] Scott, K. and Turnlund, J. (1994) J. Nutr. Biochem. 5, 342–350.
[8] Hartter, D. and Barnea, A. (1988) J. Biol. Chem. 263, 799–805.
[9] Vulpe, C. and Packman, S. (1995) Annu. Rev. Nutr. 15, 293–322.
[10] Dunn, M., Green, M. and Leach, R., Jr. (1991) Am. J. Physiol. 261, E115–E125.
[11] Fuentealba, I., Davis, R., Elmes, M., Jasani, B. and Haywood, S. (1993) Exp. Mol. Pathol. 59, 71–84.
[12] Frieden, E. (1970) Nutr. Rev. 28, 87–91.
[13] Yokoi, K., Kimura, M. and Itokawa, Y. (1991) Biol. Trace Elem. Res. 29, 257–265.
[14] Yu, S. and Beynen, A. (1994) Biol. Trace Elem. Res. 472, 71–79.
[15] Johnson, M. and Murphy, C. (1988) Am. J. Clin. Nutr. 47, 96–101.
[16] Beard, J., Connor, J. and Jones, B. (1993) Nutr. Rev. 51, 157–170.
[17] Lozoff, B. and Brittenham, G. (1986) Prog. Haematol. 14, 23–53.
[18] Youdim, M. and Riederer, P. (1993) J. Neural Transm. Suppl. 40, 57–67.
[19] Oestreicher, E., Sengstock, G., Riederer, P., Olanow, C., Dunn, A. and Arendash, G. (1994) Brain Res. 660, 8–18.
[20] Morita, H., Ikeda, S., Yamamoto, K., Morita, S., Yoshida, K., Nomoto, S., Kato, M. and Yanagisawa, N. (1995) Ann. Neurol. 37, 646–656.
[21] Yoshida, K., Furihata, K., Takeda, S., Nakamura, A., Yamamoto, K., Morita, H., Hiyamuta, S., Ikeda, S., Shimizu, N. and Yanagisawa, N. (1995) Nature Genetics 9, 267–272.
[22] Harris, Z., Takahashi, Y., Mijajima, H., Serizawa, M., MacGillivray, R. and Gitlin, J. (1995) Proc. Natl. Acad. Sci. USA 92, 2539–2543.
[23] Mearrick, P. and Mistilis, S. (1969) J. Lab. Clin. Med. 74, 421–426.
[24] Linder, M. and Munro, H. (1973) Enzyme 15, 111–138.
[25] Taylor, E., Crowe, A. and Morgan, E. (1991) J. Neurochem. 57, 1584–1592.

[26] Lee, S., Lancey, R., Montaser, A., Madanl, N. and Linder, M. (1993) Proc. Soc. Exp. Biol. Med. 203, 428–439.
[27] Kaldor, I. (1954) Aust. J. Biol. 32, 795–800.
[28] Fisher, R. (1949) The Design of Experiments, Oliver and Boyd, Edinburgh.
[29] Crowe, A. and Morgan, E. (1992) Brain Res. 592, 8–16.
[30] Bremner, I. and Young, B. (1981) Proc. Nutr. Soc. 40, 68A.
[31] Gordon, D., Leinart, A. and Cousins, R. (1987) Am. J. Physiol. 252, E327–E333.
[32] Vargas, E., Shoho, A. and Linder, M. (1994) Am. J. Physiol. 267, G259–G269.
[33] Sarkar, B. (1974) in Metal Ions in Biological Systems (Sigel, H., ed.), Vol. 12, pp. 233–281, Marcel Dekker, New York.
[34] Saenko, E., Yaropolov, A. and Harris, E. (1994) J. Trace Elem. Exp. Med. 7, 69–88.
[35] Orena, S., Goode, C. and Linder, M. (1986) Biochem. Biophys. Res. Commun. 139, 822–829.
[36] Barnes, G. and Frieden, E. (1984) Biochem. Biophys. Res. Commun. 125, 157–162.
[37] Bremner, I. (1987) J. Nutr. 117, 19–29.
[38] Crowe, A. and Morgan, E. (1996) J. Nutr. (in press).
[39] Taylor, E. and Morgan, E. (1990) Dev. Brain Res. 55, 35–42.