Copper Metabolism in Mottled Mouse Mutants

DEFECTIVE PLACENTAL TRANSFER OF ^{64}Cu TO FOETAL BRINDLED (Mo^{br}) MICE

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Decreased placental transfer of ^{64}Cu into both Mo^{br}/Y and $Mo^{br}/+$ foetuses was apparent after its administration to pregnant $Mo^{br}/+$ mice. The severity of impairment and the degree of retention and accumulation of ^{64}Cu in the placenta was not as great as has been previously observed in the gut of suckling Mo^{br}/Y mice.

The gene brindled is an allele at the sex-linked locus mottled (Mo) of the house mouse (*Mus musculus*) (Fraser *et al.*, 1953). Hemizygous mutant males (Mo^{br}/Y) die at around 14 days *post partum*, whereas heterozygous females ($Mo^{br}/+$) are viable and fertile. These mice were shown to be a model for the human sex-linked congenital copper-deficiency disease Menkes' syndrome (Hunt, 1974), and the similarities are so great that the human and mouse mutations appear homologous (Danks, 1977).

Severe malabsorption of copper by suckling Mo^{br}/Y mice results in high copper concentrations in gut and severe copper deficiency of internal organs (Camakaris *et al.*, 1979; Evans & Reis, 1978; Mann *et al.*, 1979a). As Mo^{br}/Y and $Mo^{br}/+$ foetuses have elevated placental copper concentrations and copper deficiency of internal organs (Camakaris *et al.*, 1979), it seems that they also suffer from defective transfer of copper *in utero*. In order to test this, the transfer of ^{64}Cu to foetuses of pregnant $Mo^{br}/+$ mice was observed at serial time intervals after its administration to the mothers.

Experimental

Original breeding pairs of our Mo^{br} strain were kindly supplied by Dr. David Hunt, Department of Plant Biology and Microbiology, Queen Mary College, University of London.

Adult mice were fed *ad libitum* on Mecon rat and mouse cubes (Gelman Clemco, Artarmon, N.S.W., Australia), which contained $16.7\mu\text{g}$ of copper/g dry wt. and acidified water containing $2.5\mu\text{g}$ of copper/ml. The mice were housed in plastic boxes with steel tops.

$^{64}\text{CuCl}_2$ (half-life 12.8h) was purchased from the Australian Atomic Energy Commission, Lucas

Heights, N.S.W., Australia. The radioactivity of samples was measured by using an LKB Ultra-gamma 1280 counter automatically correcting for isotope decay and background radioactivity.

Copper concentrations were determined by the method of Stevens (1972) using a Varian-Techtron CRA-90 atomic absorption spectrophotometer incorporating the BC-6 background subtract module.

$Mo^{br}/+$ mice were mated with normal male ($+/Y$) mice to produce pregnancies where equal proportions of $+/Y$, $+/+$, Mo^{br}/Y and $Mo^{br}/+$ foetuses would be expected.

Pregnant $Mo^{br}/+$ mice of at least 18 days gestation each received an intracardiac injection of $20\mu\text{l}$ of CuCl_2 in iso-osmotic 0.9% NaCl (Mann *et al.*, 1979b). After the desired length of time, they were anaesthetized with diethyl ether. Blood was collected from the ventral neck region after cutting the major blood vessels, then the liver, kidneys, foetuses and placentae were removed. All were placed in tubes and the radioactivity was measured.

After the radioactivity of each foetus had been measured, the sex of each was determined. The liver of each foetus was then removed and its copper concentration determined. Liver copper concentrations have been shown to be reliable for determining the genotype of foetuses in pregnant $Mo^{br}/+$ mice. The livers of Mo^{br}/Y and $Mo^{br}/+$ foetuses are copper-deficient (Camakaris *et al.*, 1979).

Results

The distribution of ^{64}Cu observed in pregnant $Mo^{br}/+$ mice (Table 1) showed similar trends to those observed in adult $+/Y$ and Mo^{br}/Y mice (Mann *et al.*, 1979b). However, on further comparison the initial amount of ^{64}Cu in liver and the amounts in kidney were somewhat lower than might

have been expected. This may reflect an alteration of copper metabolism during pregnancy.

In both male and female foetuses, liver copper concentrations fell into two discrete classes and genotypes were assigned accordingly (Table 2).

Figs. 1(a) and 1(b) show that the amount of ^{64}Cu in all foetuses increased in an approximately linear fashion over the 56 h after administration. However, the rate of increase in Mo^{br}/Y and $Mo^{br}/+$ foetuses was only approximately one-third of the observed in $+/Y$ and $+/+$ foetuses.

Placental content of ^{64}Cu stabilized very quickly and remained fairly constant over the 56 h, that in Mo^{br}/Y and $Mo^{br}/+$ foetuses being approximately double that in $+/Y$ and $+/+$ foetuses.

Discussion

The results of the present experiments indicate an impairment of placental transfer of copper into both Mo^{br}/Y and $Mo^{br}/+$ foetuses. This disturbance of copper transport must be the consequence of foetal contribution to the placenta, as the maternal ($Mo^{br}/+$) contribution would be the same for all genotypes. Presumably even the endothelial cells of the capillaries of the placenta show a disturbance of

copper transport similar to that seen in the mucosal cells of the gut postnatally (Evans & Reis, 1978; Camakaris *et al.*, 1979; Mann *et al.*, 1979b), although less severe.

The degree of retention and accumulation of ^{64}Cu in the placenta of Mo^{br}/Y foetuses was not nearly as great as has been seen in the gut of suckling Mo^{br}/Y mice (Evans & Reis, 1978), and this is

Fig. 1. Distribution of ^{64}Cu in foetuses and placentae of pregnant $Mo^{br}/+$ mice

Five pregnant $Mo^{br}/+$ mice each received an intracardiac injection of $^{64}\text{CuCl}_2$ ($154\ \mu\text{Ci}$; $20\ \mu\text{g}$ of Cu) and were killed after 0.5, 11, 20, 43 and 56 h respectively. Each foetus and its placenta was removed and the radioactivity of each was measured. (a) Males: $\blacksquare$, $+/Y$ foetuses; $\bullet$, placentae; $\square$, Mo^{br}/Y foetuses; $\circ$, placentae. (b) Females: $\blacksquare$, $+/+$ foetuses; $\bullet$, placentae; $\square$, $Mo^{br}/+$ foetuses; $\circ$, placentae.

Table 1. Distribution of ^{64}Cu in pregnant $Mo^{br}/+$ mice
Five pregnant $Mo^{br}/+$ mice each received an intracardiac injection of $^{64}\text{CuCl}_2$ ($154\ \mu\text{Ci}$; $20\ \mu\text{g}$ of Cu) and were killed after 0.5, 11, 20, 43 and 56 h respectively. Radioactivity in blood is expressed as a percentage of administered dose/ml and in whole liver and kidneys as a percentage of administered dose.

Time after injection (h)	^{64}Cu distribution (%)		
	Blood	Liver	Kidneys
0.5	5.6	33.8	4.5
11	1.1	17.9	8.6
20	1.2	10.2	7.4
43	1.1	9.2	7.6
56	0.5	6.5	4.4

Table 2. Copper concentrations in foetal livers

The genotype of each foetus was assigned on the basis of sex and liver copper concentration. Copper concentrations are expressed as $\mu\text{g/g}$ dry wt. of tissue. Results are mean $\pm$ s.d. followed by the number (n) and range of observations.

	Foetal genotype	[Liver copper]	
		($\mu\text{g/g}$ dry wt. of tissue) (n)	Range
Males	$+/Y$	99.1 ± 6.6 (9)	(92.7–109.9)
	Mo^{br}/Y	43.2 ± 9.5 (7)	(33.9–55.6)
Females	$+/+$	103.7 ± 16.0 (5)	(90.9–125.8)
	$Mo^{br}/+$	37.4 ± 7.3 (11)	(26.3–46.0)

consistent with the copper concentrations measured in these organs (Camakaris *et al.*, 1979). This is probably because of the different conditions pertaining to the flow of blood in the placenta compared with the flow of luminal contents in the gut. In the gut of suckling *Mo^{br}/Y* mice, unabsorbed copper remains in the luminal contents and increases in concentration along the length of the small intestine, owing to the absorption of other nutrients. A resultant mass-action effect combined with pinocytosis would then play an important role in the accumulation of very large amounts of copper in mucosa of the distal small intestine (Camakaris *et al.*, 1979).

Comparison of these observations with the situation in Menkes' syndrome is difficult, as the only human observations are of three affected fetuses at mid-gestation who, in contrast with foetal mice at late gestation, were found to have high concentrations of copper in extrahepatic tissues (Heydorn *et al.*, 1975; Horn & Heydorn, 1977). This might be thought to indicate that transfer of copper is normal in the affected human foetus. However, the known tendency of mutant cultured cells and tissues of humans and mice to accumulate copper when it is available (Goka *et al.*, 1976; Camakaris *et al.*, 1980; Mann *et al.*, 1979a,b) could produce these results even in the presence of decreased placental transfer.

The impairment of placental transfer does not seem to have any grave effect upon foetal development in either *Mo^{br}/Y* or *Mo^{br}/+* mice. This is evident from the reasonably normal weights and growth rates of *Mo^{br}/Y* mice up to 1 week of age (Mann *et al.*, 1979a; Yajima & Suzuki, 1979) and the normal viability of *Mo^{br}/+* mice. Furthermore, copper therapy at 7 days of age results in healthy

fertile adult *Mo^{br}/Y* mice (Mann *et al.*, 1979a). The foetal death of males affected by the alleles mottled (*Mo*) (Fraser *et al.*, 1953) and dappled (*Mo^{dp}*) (Phillips, 1961) indicates that placental transfer as well as the metabolism of copper by the foetus after transfer is more seriously affected in these mice.

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