Defect of Intestinal Mucosal Iron Uptake in Mice with Hereditary Microcytic Anemia (36720)

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(Introduced by R. M. Bannerman)

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Hereditary microcytic anemia of the mouse (gene symbol *mk*), is an autosomal recessive trait which was first recognized at the Jackson Laboratory in 1964 by Nash and coworkers (1). The red cell hypochromia and microcytosis which characterizes the trait is present from Day 15 of gestation to adulthood and the red cell counts of affected animals are as high or higher than those of normal animals. Since Russell and colleagues (2) have shown that *mk* assorts independently of alleles at the Hbb (hemoglobin β chain structural) locus and the Hba (hemoglobin α chain structural) locus, hereditary microcytic anemia can hardly be the result of an abnormality of hemoglobin structure. Evidence of iron deficiency in the form of depleted body stores, hyposideremia, increased total iron binding capacity and increased free erythrocyte protoporphyrin concentration has been found by Bannerman and associates (3). However, the failure to find either rapid iron clearance and high utilization of tracer doses of ^{59}Fe or a complete response to parenteral iron treatment indicated that simple iron deficiency was not the cause of the anemia. Since it was suggested that a generalized impairment of cellular uptake of iron, involving both intestinal absorption and the transfer of iron from the plasma to the erythroblast, might provide a unitary explanation for hereditary microcytic anemia (3), studies of intestinal iron absorption were undertaken.

Methods. Normal (+/+) mice and anemic (*mk/mk*) litter mates of the SEC/1 Re inbred strain, 80–150 days old, were produced at the Jackson Laboratory and transferred to Buffalo where they were housed in plastic cages and supplied with Rockland "complete" mouse diet and tap water.

In vivo iron absorption was measured by determining the percentage retention of an intragastrically administered dose (1 μg Fe) of ^{59}Fe labeled ferrous citrate by means of whole body counting in a large volume gamma scintillation counter as described previously (4). Three separate experiments were performed using 7–10 normal and anemic mice in each.

In vitro iron transport by everted duodenal loops was studied using a modification of the method of Dowdle, Schacter and Schenker (5) as previously described (6). The loops were incubated in a medium containing ^{59}Fe ferrous citrate for 3 hr. At the end of the period the loops were removed and their contents drained into plastic tubes. The total loop contents or final inside fluid (I), and a 0.1 ml aliquot were counted to determine the ^{59}Fe concentration and the final volume. Approximately 80–120% of the initial volume of inside fluid was recovered from each loop at the end of the experiments and there was no difference in recovery between loops prepared from normal and anemic animals. One milliliter of the final outside medium (O) was also counted and the muscosal uptake of iron was determined from the difference between the counts in the outside fluid before and after incubation. The accumulation of iron in the inside or loop fluid was expressed in two ways. Firstly, as the ratio of the final concentration of ^{59}Fe in the inside fluid to that in the outside fluid, and secondly, as the net transfer of iron to the inside fluid.

Six separate *in vitro* iron transport experi-

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TABLE I. Results of *in Vivo* and *in Vitro* Intestinal Iron Absorption.

	In vivo studies			*In vitro* studies			
	Percentage retention of iron			Mucosal iron uptake (ng)		Net iron transfer (ng/ml)	
Expt.	Normal	Anemia	Expt.	Normal	Anemic	Normal	Anemic
1	39.4	21.6	1	166.4	93.8	32.2	34.7
	11.7	3.6		150.4	82.8	47.3	76.8
	44.9	3.1		135.2	76.2	22.0	43.9
	41.5	26.0		140.8	84.0	32.4	42.8
	10.0	18.8	2	179.4	137.9	42.8	133.4
	79.1	28.1		216.6	159.2	114.9	233.1
	70.1	40.3		199.5	140.9	57.5	99.5
	34.8	17.8		198.2	155.7	101.2	157.7
2	43.2	2.6	3	72.2	16.8	11.2	23.1
	61.9	40.3		75.1	25.5	18.0	19.4
	46.4	9.3		93.9	18.2	25.7	26.8
	36.0	49.4		65.3	43.0	16.8	16.6
	42.1	13.6	4	108.6	69.9	23.3	79.4
	50.7	24.9		114.7	90.7	19.0	26.5
	46.7	18.8		104.7	79.5	13.1	17.8
	54.0	34.2			60.4		37.4
	19.1	37.8	5	118.1	32.8	35.3	28.9
	29.5	22.1		129.0	50.0	31.2	39.1
3	25.9	9.1		137.7	35.5	36.0	33.8
	30.8	24.8		124.1	61.4	19.9	31.9
	22.3	13.5	6	103.2	119.4	6.2	19.6
	41.2	8.5		140.3	109.8	8.4	36.3
	28.0	9.4		139.0	119.5	9.9	24.8
	28.9			142.1		16.1	
	21.5						
Mean	39.2	20.8		117.1	70.4	16.5	27.1
SE	3.5	2.7		5.3	7.0	2.2	3.4
p[a]	<.01			<.001		<.02	

[a] Analysis by the t test.

ments were performed using 3–4 normal and anemic mice in each.

Results. The results of the *in vivo* studies of intestinal iron absorption are shown in Table I. Although there was some overlap between individual anemic and normal percentage retention values, the mean value of 20.8 ± SE 2.7 for the anemic animals was significantly lower than the mean value of 39.2 ± 3.5 for the normal animals (t (46) = 3.419, $p < .01$). When each *in vivo* experiment was considered separately, 13 of the 23 (or 57%) of the percentage retention values for the anemic mice overlapped with those for the normal mice.

The results of the *in vitro* studies of intestinal iron absorption are shown in Table I. The mucosal iron uptake by the loops prepared from the anemic animals (mean 70.4 ng ± 7.0) was substantially less than that by the loops prepared from the normal animals (mean 117.1 ng ± 5.3). Although there was considerable interexperiment variation, within single experiments there was only one instance of overlap between values for the mucosal iron uptake of the anemic animals with the normal animals. A paired t test comparing the normal and anemic mean values for each separate experiment showed them to be significantly different (t (5) = 6.35, $p < .005$). Furthermore, a t test comparing all the individual normal and anemic

values also showed them to be significantly different (t (44) = 5.77, $p < .001$).

The net transfer of iron to the inside of the loops prepared from the anemic animals (mean 27.1 ng/ml ± 3.4) was greater than that for the loops prepared from the normal animals (mean 16.5 ng/ml ± 2.2). Examination of the data (see Table I) experiment by experiment showed that 11 out of the 23 (or 48%) of the values for the anemic animals overlapped with those for the normal animals. A t test comparing all the individual normal and anemic values showed them to be significantly different (t (44) = 2.65, $p < .02$).

Discussion. The *in vivo* iron absorption studies show that *mk/mk* anemic mice have a low percentage retention of intragastrically administered radioiron. There are two reasons for supposing that this low retention is the probable cause of the iron deficiency previously found in *mk/mk* animals (3). Firstly, in genotypically normal mice made iron deficient by dietary means the intestinal absorption of iron increases to a level considerably higher than that of iron replete normal mice. Thus, although the percentage retention of iron by the *mk/mk* animals is only slightly lower than that of the normal animals, it is inappropriately low when it is considered that the anemic animals are iron deficient (3). Secondly, although the difference in the percentage retention of iron between the normal and anemic animals when measured once, in a single test, is not remarkably different, the lower retention by the anemic animals when considered over a prolonged period of time might be expected to lead to iron deficiency.

The most striking result of the *in vitro* studies of iron transport is the low mucosal iron uptake by the anemic mice when each individual experiment is considered separately. The interexperiment variation can probably be attributed to variables such as atmospheric conditions, loop dissection time and mouse age.

The combination of iron deficiency and iron malabsorption exists in sex-linked anemia (gene symbol *sla*). However, in contrast to sex-linked anemia, in which there is a defect in the transfer of iron from the mucosa to the serosa (6), in hereditary microcytic anemia transfer appears to be enhanced although mucosal uptake is reduced. It is assumed that this enhancement of mucosa to serosa iron transfer is a compensatory phenomenon. Presumably *in vivo* this compensatory increase in mucosa to serosa transfer is not sufficient to overcome the defect in mucosal uptake.

The finding of impaired mucosal iron uptake in hereditary microcytic anemia lends support to the hypothesis put forward by Bannerman and associates (3) that a generalized impairment of cellular uptake of iron might provide a unitary explanation for the anemia.

To our knowledge this is the first time that a genetically determined defect in the mucosal uptake of iron has been described. The demonstration that two anemia-producing mutations in the mouse, the autosomal *mk* and the X-linked *sla,* determine defects of intestinal iron absorption at different steps, provides two valuable models for the study of the still obscure mechanisms controlling iron absorption in both the mouse and man.

Summary. Intestinal iron absorption was studied in mice with hereditary microcytic anemia (gene symbol *mk*), an autosomal recessive trait characterized by hypochromia and microcytosis. *In vivo* studies by means of whole body counting following the intragastric administration of radioiron showed impaired intestinal absorption of iron. Further *in vitro* studies using the everted duodenal loop technique, demonstrated a defect in the mucosal uptake of iron. Hereditary microcytic anemia should, therefore, provide a valuable model for the study of the mechanisms controlling intestinal iron absorption.

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