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John A. Edwards, Robin M. Bannerman

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Research Article

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Hereditary Defect of Intestinal Iron Transport in Mice with Sex-Linked Anemia

JOHN A. EDWARDS and ROBIN M. BANNERMAN

From the Medical Genetics Unit, Department of Medicine, State University of New York at Buffalo and Buffalo General Hospital, Buffalo, New York 14203

ABSTRACT Iron transport by everted duodenal sacs in vitro was studied in mice with sex-linked anemia (gene symbol *sla*) (an inherited iron deficiency anemia), in normal mice, and in normal mice on iron-deficient and iron supplemented diets. Although the over-all mucosal uptake of iron was the same in *sla* and normal sacs, transport of iron to the inside of the sac was much decreased in *sla*. The iron transport defect in *sla* was emphasized by the fact that genotypically normal mice on an iron-deficient diet demonstrated greatly increased iron transport. Electrophoretic analysis of protein extracted from *sla* and normal sacs showed only one iron-binding fraction. The *sla* and normal fractions had the same mobility and corresponded in position to the major band of horse ferritin.

It thus appears that the iron deficiency of *sla* is due to a genetically determined defect in mucosal iron transport and that this defect is not associated with any demonstrable abnormality of a major iron-binding protein.

INTRODUCTION

In vivo studies in sex-linked anemia of the mouse (gene symbol *sla*), an inherited iron deficiency anemia, have shown a defect in iron absorption (1, 2). To define the nature of this defect, in vitro iron transport by everted duodenal sacs was studied using a modification of the method of Dowdle, Schachter, and Schenker (3).

METHODS

Iron transport was studied in four groups of male mice aged 50–100 days.

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Group 1. Normals fed standard mouse diet.
Group 2. Anemics fed standard mouse diet.
Group 3. Normals fed an iron-deficient diet (4).
Group 4. Normals fed the same iron-deficient diet supplemented with 100 mg ferrous ammonium sulfate per 100 g of diet.

The mice in groups 1 and 2 were a first backcross to the C57BL/6 Jax strain of a hybrid between this strain and the original mixed strain in which the mutation arose. The mice in groups 3 and 4 were C57BL/6 Jax strain.

The mice were fasted, with water supplied, for 18 hr before an experiment and then killed by cervical traction. The intestine was divided at the duodenopyloric junction and 4 cm of gut, comprising duodenum and adjacent jejunum, were dissected free and removed. The segment was everted over a glass capillary tube and immediately chilled in ice-cold 0.146 M NaCl–0.004 M KCl. One end of the segment was cannulated with a polythene tube attached to a 1 ml syringe and the other end ligatured. The segment was filled with 0.2 ml of a freshly prepared medium containing 0.145 M NaCl, 0.0001 M CaCl₂, 0.04 M D-mannose, 0.004 M Tris buffer (pH 7.4), and 0.008 M sodium ascorbate. The sac preparation was completed by ligaturing the other end of the gut segment as the polythene tube was withdrawn. The sacs were placed in 25-ml Erlenmeyer flasks containing 8 ml of the same medium plus sufficient ⁵⁹Fe ferrous citrate to give 40,000 counts/min per ml in a large volume gamma scintillation counter. The flasks were gassed with a continuous stream of oxygen at a flow rate of 1 liter/min while incubation proceeded in a shaking water bath at 37°C for 3 hr. The sacs were then removed and their contents drained into plastic tubes. The total sac contents or final inside fluid (I), and a 0.1 ml aliquot were counted to determine the ⁵⁹Fe concentration and the final volume. Approximately 80–120% of the initial volume of inside medium was recovered from each sac at the end of the experiments and there was no difference in recovery between normal and *sla* sacs. 1 ml of the final outside fluid (O) was also counted and the mucosal uptake of iron determined from the difference between the counts in the outside fluid before and after incubation. The accumulation of iron in the inside or sac fluid (I) was expressed in two ways. Firstly, as the ratio of the final concentration of ⁵⁹Fe in the inside fluid to that in the outside fluid, and secondly, as the net transfer of iron to the inside fluid.

At the end of one experiment four normal and four *sla* sacs were pooled and then homogenized by hand in 20 ml

TABLE I
Results of the Everted Duodenal Loop Experiments

Group	Animals	Number	Mean Hb level <i>g/100 ml</i>	Mean I/O ratio \pm SE	Net transfer of Fe to inside medium <i>mμg/ml</i>	P*
1	Normal male	12	13.9	3.66 \pm 1.16	52.5 \pm 17.2	<0.01
2	<i>sla</i> male	12	6.1	0.75 \pm 0.11	12.4 \pm 1.8	
3	Iron-deficient diet male	4	13.6	22.36 \pm 1.60	190.9 \pm 22.8	<0.001
4	Iron-supplemented diet male	4	15.9	1.04 \pm 0.26	9.1 \pm 1.4	

Hb = hemoglobin.
* Analysis by the paired *t* test.

of 0.01 M NaCl in a glass-Teflon homogenizer. The homogenates were centrifuged at 4°C for 20 min at 2500 rpm. The protein in the supernatant was precipitated by saturation with ammonium sulfate. The precipitated protein was collected by centrifugation, dissolved in the minimum amount of distilled water, dialyzed extensively against Tris-EDTA-borate buffer (pH 8.6), and subjected to vertical starch-gel electrophoresis in the same buffer. Each sample, and horse ferritin, which was used as a control, was run in duplicate. Following electrophoresis, one section of the gel was cut into 1 mm slices along the axis of migration and the ⁵⁹Fe activity in each slice counted; the other section was stained for protein using amido black.

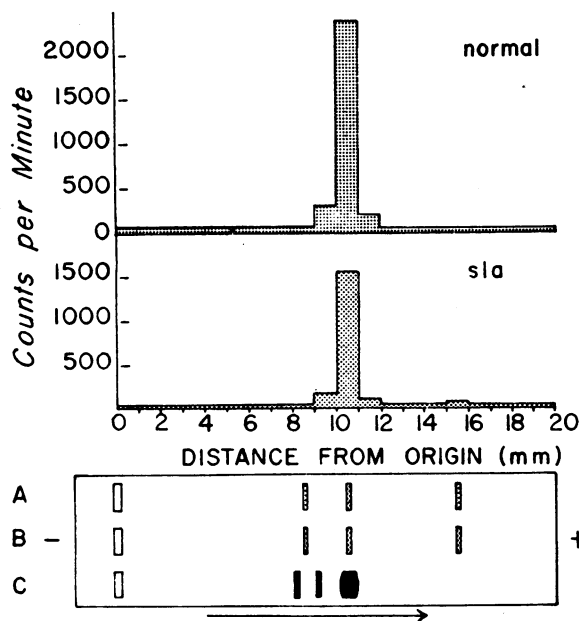


FIGURE 1 Vertical starch-gel electrophoresis in Tris-EDTA-borate buffer (pH 8.6) of (A) duodenal protein from normal mice, (B) duodenal protein from mice with sex-linked anemia (*sla*), and (C) horse ferritin. The gel was cut into 1 mm slices along the axis of migration and the radioactivity in each slice measured in a large volume gamma scintillation counter. Anode (+), cathode (-).

RESULTS

In normal mice fed standard diet (group 1) the I/O ratio was always greater than 1, demonstrating that transport of iron had occurred against a concentration gradient. The mean I/O ratio for this group was 3.66 and the mean net transfer of iron was 52.5 μ g/ml (Table I). In contrast, the I/O ratio for the anemic mice (group 2) failed to reach 1 and the mean net transfer of iron was only 12.4 μ g/ml. In genotypically normal mice fed an iron-deficient diet (group 3) there was a striking augmentation in iron transport although the mice had not yet become anemic. Both the mean I/O ratio (22.36) and the mean net transfer of iron (220.0 μ g/ml) of this group were much higher than the corresponding values for both the normal mice fed an iron-supplemented diet (group 4) and the mice with sex-linked anemia (group 2).

There was no significant difference between the overall mucosal uptake of iron by the normal and *sla* sacs, both types of sac taking up approximately 220 μ g.

The electrophoretic analysis of the duodenal protein from normal and *sla* sacs revealed only one peak of ⁵⁹Fe activity (Fig. 1). There was an exact correspondence between the position of the normal ⁵⁹Fe peak, the position of the *sla* ⁵⁹Fe peak, and the position of the major band of horse ferritin.

DISCUSSION

The results of these in vitro studies of iron transport confirm the malabsorption of iron in sex-linked anemia previously demonstrated in vivo. They show that the genetically determined defect in *sla* is one of impaired transport of iron across the mucosa rather than an impairment of mucosa uptake of iron. Similar results have been reported recently in abstract by Manis (5). The severity of the iron transport defect in mice with sex-linked anemia, which are iron deficient, is emphasized by the fact that in genotypically normal iron-deficient mice which are not yet anemic there is a

marked increase in iron transport. The results of the electrophoretic analysis of duodenal protein show that the iron transport defect in *sla* is not associated with any demonstrable abnormality of a major mucosal iron-binding protein.

Further studies of iron transport in sex-linked anemia may help to elucidate the role of the gene product of the normal allele in iron absorption, and help to clarify the still obscure mechanisms controlling intestinal iron absorption in both the mouse and man.

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