Effect of Chronic Nutritional Iron Deficiency on the Small Intestinal Disaccharidase Activities of Growing Dogs (33615)*

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(Introduced by E. E. Baker)

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Chronic severe iron deficiency in infancy and early childhood may be associated with duodenal and jejunal mucosal atrophy (1, 2), impaired absorption of fat, D-xylose (1, 2), and hemoglobin iron (3). Puppies with severe iron deficiency, like children with severe iron deficiency, may malabsorb hemoglobin iron and fat (3). The results of the present study demonstrate additional abnormalities of the small intestinal mucosa, namely, decreased disaccharidase activity associated with chronic iron deficiency in the growing pup.

Materials and Methods. Five litters (4 Weimaraner, one beagle) each of four puppies, were weaned at 35 days and subsequently fed a powdered standard dog diet containing 10 mg of iron/100 gm diet (General Biochemicals, Chagrine Falls, Ohio). Two members of each litter received an identical diet with the exception that the iron content was 0.09 mg/100 gm diet (3). After approximately 8 weeks on the diet, venous blood samples were drawn for preparation of a stained peripheral blood film, estimation of hemoglobin, and measurement of serum iron and total iron-binding capacity. The animals were anesthetized with nembutal, the jejunum removed and immediately chilled to 4° in cold physiological saline. After a thorough wash with cold saline, the mucosa was scraped from the serosa on a glass tray maintained at 4°. The mucosal scrapings were homogenized in cold physiological saline (1 gm to 9 ml) using a Potter-Elvejehm homogenizer. In some experiments, a portion of the mucosal scrapings was used for preparation of intestinal brush borders (4). Assays of lactase, sucrase, and maltase were carried out according to Dahlqvist (5). In iron-replacement studies, disaccharidase activities were determined in homogenates from iron-deficient puppies to which iron as ferric chloride (2 μg Fe^{3+}/cc homogenate) was added. In other experiments desferrioxamine (21.5 μg/cc homogenate) was added to control homogenates to chelate inorganic iron in vitro. Jejunal mucosal folate conjugase[1] was estimated by incubating mucosal homogenate with folate polyglutamate substrate in phosphate citrate buffer, pH 4.6, containing 1 gm/100 ml of ascorbic acid. "Free" folate released was measured by microbiological assay with *Lactobacillus casei* as test organism. Details of the method will be described elsewhere (Hoffbrand *et al.*, 1968, to be published). Protein was estimated by the method of Lowry *et al.* (6), serum iron by the method of Caraway (7), and hematological methods were standard (8).

Results. Evidence of iron deficiency. This was provided by the presence of microcytic hypochromic erythrocytes in the stained peripheral blood films of the iron-deficient but not in the films of the control puppies. In addition the hemoglobin concentrations of the iron-deficient puppies ranged from 2.6 to 6.8 gm/100 ml (mean 4.5 gm/100 ml), whereas those of the control puppies ranged

[1] Folate conjugase splits folate polyglutamates into folate monoglutamate. The latter supports the growth of *L. casei* and are termed "free." The former does not support the growth of *L casei.*

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TABLE I. Disaccharidase Activities of Jejunal Mucosal Homogenates from Iron-Deficient and Control Puppies.

	Lactase (μmoles lactose hydrolyzed/min/g protein)		Sucrase (μmoles sucrose hydrolyzed/min/g protein)		Maltase (μmoles maltose hydrolyzed/min/g protein)	
Animal pair	Control	Deficient	Control	Deficient	Control	Deficient
1	16.5	8.4	50.1	25.4	168.1	96.7
2	22.9	10.1	37.9	31.3	194.0	103.8
3	19.8	9.0	38.1	28.5	160.7	119.8
4	1.0	—	6.1	3.6	23.8	23.9
5	21.7	6.8	94.4	50.5	353.5	188.3
Mean ± SE	16.4 ± 4.0	6.9 ± 1.8[a]	45.3 ± 14.5	27.7 ± 7.5[b]	180.0 ± 52.6	106.5 ± 26.3[c]
Iron-deficient as % of control		42.1		61.1		59.2

[a] t test of paired samples $p < 0.01$.
[b] $p < 0.04$.
[c] $p < 0.03$.

from 8.0 to 10.1 gm/100 ml (mean 9.3 gm/100 ml). Moreover, the serum iron levels of the former puppies ranged from 18 to 42 μg/100 ml (mean 24 μg/100 ml) whereas those of the latter puppies ranged from 52 to 139 μg/100 ml (mean 90 μg/100 ml) and the mean total iron-binding capacity of the iron-deficient puppies was 430 μg/100 ml and of the control animals 390 μg/100 ml.

Disaccharidase activities. Disaccharidase activities of iron-deficient and control animal are listed in Table I. Mucosal homogenates from the iron-deficient group invariably had lower lactase, sucrase, and maltase activities than those from their control littermates. Mean activity for each of the disaccharidase enzymes in the iron-deficient group was approximately 50% of the control value. In brush border preparations from iron-deficient puppies lactase, sucrase, and maltase activities were similarly reduced to about one half of the control values (Table II).

Addition of inorganic iron as ferric chloride *in vitro* in sufficient quantity to bring the iron concentration of iron-deficient mucosal homogenates equal to that of the control mucosal homogenates did not increase the disaccharidase activities of iron-deficient mucosa (Table III). Nor did desferrioxamine in sufficient concentration to bind all free inor-

TABLE II. Disaccharidase Activities of Intestinal Brush Border Preparations from the Mucosa of Iron-Deficient and Control Puppies.

Animal	Lactase (μmoles lactose hydrolyzed/min/g protein)	Sucrase (μmoles sucrose hydrolyzed/min/g protein)	Maltase (μmoles maltose hydrolyzed/min/g protein)
Control A[a]	122.5	459.7	1482.1
B[a]	89.6	529.9	1181.1
Mean	106.0	494.8	1331.6
Iron-deficient C[a]	93.5	297.7	806.6
D[a]	45.1	209.8	601.1
E[a]	38.1	227.6	545.0
Mean	58.9	245.1	650.9
Iron-deficient as % of control	55.5	49.5	48.9

[a] Controls A, B and Iron-deficient C, D, E are from Animal pairs 1 and 2 (Table I).

TABLE III. Disaccharidase Activities of Jejunal Mucosal Homogenates in the Presence of Additional Iron or Desferrioxamine.

Treatment	Lactase (μmoles lactose hydrolyzed/min/g protein)	Sucrase (μmoles sucrose hydrolyzed/min/g protein)	Maltase (μmoles maltose hydrolyzed/min/g protein)
Control pup	13.6	79.9	296.6
+ Des[a]	12.8	81.9	289.8
+ Fe[b]	12.3	85.4	292.5
Iron-deficient pup	6.8	50.5	188.3
+ Des[a]	6.2	49.8	192.9
+ Fe[b]	5.3	46.8	180.9

[a] Desferrioxamine.
[b] Iron as $FeCl_3$.

ganic iron reduce disaccharidase activities in control mucosa.

Folate conjugase. In contrast to the findings with the disaccharidase enzymes, the folate conjugase activities in the iron-deficient mucosal homogenates prepared from animal pairs I, II (mean 640 ng folate released/mg protein/90 min) and their brush border preparations (mean 1110 ng folate released/mg protein/90 min) were similar to the concentrations in the corresponding control preparation (650 ng folate released/mg protein/90 min and 1040 ng folate released/mg protein/90 min respectively).

Histology. No difference in the intestinal mucosal appearance could be detected by light microscopy between the iron-deficient and control animals.

Discussion. The results here clearly show that severe nutritional iron deficiency is associated with a significant reduction in the lactase, sucrase, and maltase activities of the small intestinal mucosa of the growing dog. The mechanism for this reduction is uncertion. Iron deficiency is known to cause a reduction of the concentration of iron-containing enzymes, such as cytochrome oxidase, in the intestinal mucosa and elsewhere (9, 10). However, there is no evidence that the small intestinal disaccharidase enzymes either contain or require iron for activity. Indeed the studies of iron replacement and iron binding with desferrioxamine reported here suggest that free inorganic iron is not essential for lactase, sucrase, or maltase activities. It is more likely that the reduced disaccharidase activity of the iron-deficient mucosa is related to specific injury by severe iron deficienty of the intestinal mucosa of the growing animal. In children with severe iron deficiency, conventional microscopic and electron microscopic structural changes, as well as defects of fat, xylose, and haem absorption may be observed and are reversed by iron therapy (1–3). In the present study, morphologic changes were not seen in the mucosa by light microscopy. However, it is not unusual in children to observe functional alterations of the intestinal mucosa due to iron deficiency in the absence of morphologic change that can be detected by light microscopy (1, 2).

In contrast to the findings with the disaccharidase enzymes which are located mainly in the brush border region, folate conjugase activity was not reduced by iron deficiency. These findings suggest that whatever defect is produced by iron deficiency on the intestinal mucosa, it occurs principally in the brush border region of the intestinal epithelial cells.

Summary. Lactase, sucrase, and maltase activities were reduced to approximately 50% of controls in both jejunal mucosal homogenates and brush border preparations from puppies with chronic severe nutritional iron deficiency. *In vitro* studies failed to

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show a requirement for free inorganic iron by these enzymes. On the other hand, folate conjugase activity was similar in the jejunal mucosa of iron-deficient and control puppies. No histological abnormalities were noted in the mucosa of the iron-deficient animals. It is suggested that iron deficiency may cause a functional impairment in the brush border region of the growing animal before structural changes occur.

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Blockade of Ovulation in the Rabbit with Catecholamines and Sympathomimetics* (33616)

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The literature dealing with pharmacologic agents that block ovulation in various species of animals was reviewed by Everett in 1961 (1). Although a variety of drugs which influence the central or autonomic nervous systems have the ability to inhibit both "spontaneous" and reflexogenous" ovulation, they do not appear to be pharmacologically similar to one another. This fact is increasingly obvious when one considers that parasympatholytic compounds (such as atropine and Banthine) (1–4), adrenergic blocking agents (such as Dibenamine) (5–14), as well as reserpine and chlorpromazine (15, 16) all block ovulation under certain conditions.

Many pharmacologic agents which are effective in blocking ovulation can also produce marked disruption of the sympathetic nervous system. Phenoxybenzamine (Dibenzyline), a representative of the beta haloalklyamine family, blocks the uptake of exogenous norepinephrine and endogenous epinephrine (17, 18). Two hours after administration of Dibenamine, the tissue levels of catecholamines are increased (19). Both reserpine and chlorpromazine are effective in blocking ovulation. The pharmacologic properties of reserpine reside in its ability to deplete the stored catecholamines of the sympathetic nervous system (20–22). Similarly, chlorpromazine has an effect on the catecholamines: it increases both the tissue levels of catecholamines and their metabolism (19, 23). Atropine favors the expression of

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