Effect of Inosine on Iron Absorption in Rats.* (25403)

BARBARA CHENEY AND CLEMENT A. FINCH
Dept. of Medicine, University of Washington School of Medicine, and King County Central Blood Bank, Seattle, Wash.

In a series of studies Mazur and his collaborators(1) presented data to indicate that iron may be mobilized from tissue cells through reduction and liberation of ferritin iron by xanthine oxidase. This mechanism may be activated through initial reduction of xanthine oxidase by injected nucleosides. Mazur's studies deal chiefly with mobilization of iron from existent tissue stores, and the effect of injected inosine on transport of iron across intestinal mucosa is examined.

Materials and methods. Iron absorption as affected by inosine, was studied in male Sprague-Dawley rats weighing 200 to 300 g. All animals were given 5 mg of iron intramuscularly (Imferon†) 10 to 14 days before experiments to eliminate the possible influence of iron deficiency on our results. Approximately 10 μc of Fe^{59} as $FeCl^3$ in volume of 0.5 ml of acidified saline was administered by intubation to the etherized rat after 24 hour fast. Inosine‡ was dissolved in saline and injected intravenously unless otherwise stated. Animals were sacrificed by exsanguination 8 days later while under ether anesthesia. The liver was perfused free of blood. Total blood activity was determined from aliquot of blood removed and an assumed blood volume of 50 ml/kg. Tissue counting was performed in most instances by placing the tissue or its aliquot directly in a vial for counting. Carcass analysis was performed by digesting the entire autoclaved carcass in various fractions in nitric acid and analyzing an aliquot from each fraction. A scintillation counter was employed for counting and a total of at least 4,000 counts compiled on each sample. Samples were usually in counting range of over 20 times background, *i.e.*, 2,000 to 20,000 counts/minute.

Results. Absorption studies are summarized in Table I. When given intravenously, inosine had a consistent effect in increasing amount of iron absorbed. This effect was maximal when inosine and iron were administered simultaneously. Inosine was without effect if given orally. The effect of inosine injection on iron absorption, was present over a range of iron dosage (0.07 to 4.6 mg/kg). The amount of inosine required for the effect appeared to be as little as 0.03 g/kg.

Body distribution of absorbed radioiron with and without parenteral administration of inosine is shown in Table II. Liver and blood contained some 94% of total carcass activity in controls and 90% in inosine-treated animals. Our studies indicate that inosine given at time of intravenous injection of transferrin-bound iron also produces a greater deposition of iron in the carcass exclusive of blood, marrow and liver.

Discussion. Any information which provides an understanding of the intracellular handling of iron would appear important to our understanding of iron metabolism. Mazur's studies(1) or the effect of xanthine oxidase *in vivo* in releasing iron from the ferritin reservoir of liver provide a possible insight into this problem. We have been impressed with the similar and the synchronous behavior of various body cells in response to certain situations which alter iron kinetics. Thus following bleeding, the reticulo-endothelial cell, the gastrointestinal-mucosal cell and the placental cell all show an increase in their release of iron(2,3). The results here reported indicate that inosine influences iron transport across the intestinal mucosa as well as in the liver. The magnitude of this effect however was small (50 to 100% above normal absorption) and suggests that the much greater increase observed in iron depletion or in idiopathic hemochromatosis (300 to 600%) may

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† Kindly supplied by Lakeside Labs, Milwaukee, Wisc.

‡ Obtained from Schwarz Labs, Mount Vernon, N. Y. and Nutritional Biochemicals Corp., Cleveland, O.

TABLE I. Absorption Studies in Rats.*

Exp.	Condition	No. of animals	Avg wt, g	Amount Iron, mg/kg	Amount Inosine, g/kg	% uptake Blood	% uptake Liver
1 A	Controls	9	313	2.56	(Saline)	2.0 ± .73†	.30 ± .14†
B	Inosine 0°	3	323	2.78	.11	3.5 ± 1.08	.77 ± .13
C	4° before 0 time	4	312	2.56	.10	1.4 ± .39	.30 ± .05
D	2° " "	4	321	2.49	.11	2.2 ± 1.20	.38 ± .12
E	2° after 0 time	4	317	2.52	.10	2.5 ± 1.32	.63 ± .48
F	4° " "	4	339	2.36	.10	2.5 ± .93	.41 ± .19
2 A	Controls	10	347	2.31		.91 ± .24	.16 ± .02
B	Inosine 0 time	5	315	2.54	.29	1.16 ± .69	.39 ± .31
C	*Idem*	5	327	2.45	.18	1.30 ± .16	.32 ± .06
D	"	5	342	2.34	.09	1.45 ± .69	.39 ± .06
E	"	5	311	2.57	.03	1.13 ± .49	.30 ± .14
3 A	Controls	4	340	.07	.09	4.61 ± 2.69	.89 ± .46
	Inosine 0 time	4	320	.08	.09	7.84 ± 5.81	1.13 ± .75
B	Controls	4	345	.29	.09	1.09 ± .51	.23 ± .04
	Inosine 0 time	4	328	.30	.09	2.73 ± .76	.43 ± .17
C	Controls	4	332	1.51	.09	1.10 ± 1.48	.23 ± .19
	Inosine 0 time	4	332	1.51	.09	1.58 ± .36	.28 ± .09
D	Controls	3	326	4.60	.09	.89 ± .22	.14 ± .06
	Inosine 0 time	3	327	4.59	.09	.92 ± .44	.37 ± .37
4 A	Controls	8	275	.36		2.56 ± .80	.54 ± .12
B	Inosine P.O.	5	277	.36	.11	2.27 ± .58	.52 ± .09
C	*Idem*	6	318	.31	.19	2.40 ± .67	.64 ± .18

† ± stand. dev. Bessel's correction, $S = \sqrt{\frac{\Sigma x^2}{n} \times \frac{n}{n-1}}$. x = individual results

* Animals were sacrificed 8 days after administration of radioiron.

x = avg

be mediated in another manner. These data do not exclude a non-specific effect of inosine, for example, on intestinal blood flow. They merely define the positive effect of inosine on iron absorption and its magnitude. Observations on distribution of absorbed radioiron in the carcass of rats, indicate that the difference in absorption is a true difference and not dependent on a change in internal distribution of iron. They also indicate that blood and liver analyses suffice in measurements of absorption of iron in non-pregnant animals.

TABLE II. Distribution of Absorbed Iron at 8 Days.*

	Control group	Inosine*
No. of animals	6	6
Wt	337 (308–360)	338 (296–370)
	% of admin. iron	
Blood activity	4.34	4.93
Liver "	1.23	1.35
Carcass "	.36	.66
Total "	5.93	6.93

* Dose of 0.3 mg/kg of iron was given by mouth and at same time 30 mg of inosine was given intrav. Liver was not perfused.

Summary. Inosine administered intravenously at time of iron absorption increased the amount of iron absorbed by 50 to 100% in rats.

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