Biochemical Pharmacology, Vol. 19, pp. 2551–2554. Pergamon Press. 1970. Printed in Great Britain

EFFECT OF INOSINE AND ITS METABOLITES ON INTESTINAL IRON ABSORPTION IN THE RAT

A. FAELLI and G. ESPOSITO

Institute of General Physiology, University of Milan, Italy

(*Received* 16 *December* 1969; *accepted* 24 *February* 1970)

Abstract—Inosine enhances iron absorption in *in vivo* (rat, Wistar strain) and in isolated small intestine (same animal). Inosine is locally and partially metabolized to hypoxanthine, xanthine and uric acid. These metabolites, separately administered, positively affect intestinal iron absorption. Inosine metabolites may be assumed to be involved in the process of iron absorption.

THE AMOUNT of iron absorbed from the intestine is largely regulated by the requirements of the organism,[1] but some substances, such as ascorbic acid, enhance iron absorption under all conditions.[2] Inosine, a product of adenosine metabolism, has been shown to enhance iron absorption in rats when given intravenously, but to have no effect if given orally.[3] In the present paper this different behaviour was investigated.

MATERIALS AND METHODS

In vivo and *in vitro* experiments were performed. First the intestinal absorption of inosine was determined. Albino male rats (Wistar strain) weighing about 250 g and golden hamsters weighing about 150 g, starved over 24 hr, were used. Under barbituric narcosis the duodenum and the first part of the jejunum were removed and everted according to the Wilson and Wiseman technique,[4] incubated for 2 hr at 37° in 10 ml of Krebs–Henseleit-bicarbonate solution[5] added with glucose 13·9 mM, with or without inosine 20 mM, and gassed with 95% O_2 and 5% CO_2. One ml of the inosine-free solution was put into the serosal sac, which was weighed at the beginning and end of the experiment. The emptied intestine was dried at 100° for 24 hr and weighed. The mucosal and serosal fluids were deproteinized adding 1/5 in volume of 20% trichloracetic acid and then centrifuged; inosine and its metabolic products were quantitatively determined according to the following chromatographic method. A descending chromatography was carried out for 14 hr at room temperature with Whatman No. 1 filter paper; the solvent was composed of 20 parts acetic acid, 80 parts *n*-butanol and 100 parts water; only the non-aqueous phase was utilized. Spots were visualized under a Mineralight lamp, the identification based on R_f values and the eluted spots quantitatively determined with a Beckman spectrophotometer at 248 mμ.

In a second set of experiments the *in vitro* iron absorption in the presence or absence of inosine 20 mM was determined. These experiments were performed with the same intestinal tract of rats and following the Wilson and Wiseman technique[4] as in the first set, but the composition of the mucosal incubating fluid was: NaCl 145 mM,

mannose 27·8 mM, sodium ascorbate 0·8 mM, Tris buffer 5 mM, $FeSO_4$ 0·1 mM with trace amounts (100 μc/l.) of ^{59}Fe under a ferrous form (Radiochemical Centre, Amersham, England; specific activity 1100 μc/μmole) and with or without inosine 20 mM. The pH of this solution was 7·4 at 37° (Metrohom pHmeter, Switzerland). In this solution iron does not precipitate, due to the presence of sodium ascorbate. The same iron-free solution was used as a serosal fluid.

One ml of the serosal fluid was put into the serosal sac at the beginning of the experiment and the intestine was incubated for 2 hr in 25 ml of the mucosal fluid gassed with pure O_2. The intestinal sac was weighed at the beginning and end of the experiment and ^{59}Fe detected in the mucosal and serosal fluids by a scintillation spectrophotometer (Packard mod. 3003). The emptied intestine was dried at 100° for 24 hr and weighed. These data allow us to calculate the iron absorbed by the intestine from the mucosal incubating fluid and the iron transported into the serosal side.

In a third set of experiments the effect of inosine 20 mM, hypoxanthine 5 mM, xanthine 0·1 mM and uric acid 0·1 mM on iron *in vivo* absorption was investigated. Albino male rats, starved over 24 hr were used. Under barbituric narcosis the bile duct was ligated and the same intestinal tract as in other sets was cannulated. The intestinal lumen was washed and filled for three subsequent 0·5-hr periods with 1 ml of the same fluid as in the second set of experiments, with trace amounts (0·2 μc) of uniformly tritiated inulin (Radiochemical Centre, Amersham England; specific activity 250 μc/μmole). The first and the third periods were taken as controls and the luminal fluid was inosine-free, while in the second period one of the above mentioned test substances was added. At the end of each period a small sample of the luminal fluid was taken out and tritiated inulin and ^{59}Fe were separately determined by a scintillation spectrophotometer (Packard mod. 3003). Finally the emptied intestine was dried at 100° over 24 hr. Assuming that inulin is not absorbed by the intestine and knowing the fluid volume and the iron concentration in the intestine at the end of each period, we could calculate the amount of iron not absorbed and that absorbed in gram dry weight per hour for each period.

DISCUSSION

The first experiments shows that the duodenum and the first part of the jejunum, where most of the iron is absorbed, are also able to transform inosine to ribose and hypoxanthine, the latter is further metabolized to xanthine and uric acid. All these substances were present also in the serosal side: inosine 30 $\pm$ 6 μmoles/g dry weight/hr in hamster intestine and 25 $\pm$ 5 μmoles/g dry weight/hr in rat intestine; hypoxanthine 14 $\pm$ 2 μmoles/g dry weight/hr in hamster intestine. We have detected only a small quantity of xanthine and uric acid, in spite of the presence of a good amount of xanthine oxidase (an intracellular enzyme[6]) in the intestine;[7] the real quantity should be higher but not in solution, because of the low solubility of these two substances. None of these substances was present in control experiments. A good concentration of hypoxanthine (5·1 $\pm$ 0·5 mM) was present also in the mucosal fluid: this may be due to the inosine nucleosidase activity and to the hypoxanthine secretion into the intestinal lumen.[6]

Results similar to ours have been found using adenosine monophosphate as a mucosal incubating substance.[8]

The second experiments (Table 1) show that iron absorption from the mucosal

TABLE 1. EXPERIMENTS *IN VITRO*

	Number of	Iron absorption	Iron transport
Control	6	1688 ± 79	46 ± 11
20mM Inosine	6	2551 ± 145	30 ± 3

The data (with their S.E.M.) are expressed in nmoles per gram dry weight and per hour.

TABLE 2. EXPERIMENTS *IN VIVO*

	Iron absorption (nmoles g^{-1} hr^{-1})				
Number of experiments	5	3	3	6	4
1st period	79 ± 23	175 ± 59	174 ± 25	198 ± 58	190 ± 10
2nd period	193 ± 38	282 ± 30	299 ± 39	282 ± 34	184 ± 13
Test substance	inosine 20 mM	hypoxanthine 5 mM	xanthine 0·1 mM	uric acid 0·1 mM	none
3rd period	125 ± 31	235 ± 30	238 ± 11	211 ± 38	161 ± 10

The data (with their S.E.M.) are expressed in nmoles per gram dry weight and per hour.

space is enhanced by the presence of inosine in the incubating fluid. Conversely, iron transport to the serosal space does not seem to be increased by the nucleoside; this may be due to the slowness of the phenomenon and to the absence of blood stream in the intestine.

The third experiments (Table 2) shows that iron absorption is enhanced not only by inosine, but also by hypoxanthine, xanthine and uric acid. With no substance addition in the second period of incubation, iron absorption does not vary substantially.

From Tables 1 and 2 the absolute values of iron absorption appear to be higher in *in vitro* than in *in vivo* experiments; this can be accounted for by the better concentration gradient for iron absorption assured by the larger perfusing fluid (25 ml) in the second experiments, in comparison to the third (1 ml).

Three main hypotheses have been proposed on iron absorption. In the first one in order of time, iron crosses cellular membranes under a ferrous form, but it is bound under a ferric form both in cells (ferritin) and in the blood (siderophilin).[9, 10]

In the second hypothesis iron is actively absorbed and concentrated by the intestine[11] as many other actively absorbed substances (glucose etc.).

To provide a better explanation of iron absorption regulation these hypotheses have been extended by supposing a "messenger" in absorbing cells.[12]

In the third hypothesis iron is bound by low molecular weight chelators during transport and by insoluble chelators at high molecular weight in the cells.[13]

Our results do not agree with some aspects of the last two hypotheses: in fact in the second set of experiments iron was never concentrated in the serosal space and inosine, hypoxanthine, xanthine and uric acid which affect iron absorption (Table 2) are not low molecular weight chelating agents. On the contrary it is known that hypoxanthine and xanthine are oxidized in the presence of xanthine oxidase[14] and rat intestine contains a great quantity of this enzyme. Furthermore it has been demonstrated

in *in vitro* experiments than xanthine oxidase in the presence of its substrates (hypoxanthine and xanthine) and uric acid alone can reduce ferritin iron.[14] In this way ferritin iron may give rise to ferrous iron which would leave the intestinal cells and further iron could enter the cells from the lumen to be bound to apoferritin.

CONCLUSIONS

Inosine in the mucosal medium is able to cross the intestinal barrier in *in vitro* experiments and to enhance the intestinal absorption of iron. Inosine, hypoxanthine, xanthine and uric acid experimentally introduced into the duodenal tract in *in vivo* experiments enhance iron intestinal absorption. We conclude that inosine and its derivatives seem to be active also when present in the intestinal lumen only.

Acknowledgements—The authors are indebted to Dr. Chiaramonti for the chromatographic determinations of inosine and related compounds.

REFERENCES

1. W. H. CROSBY, *Am. J. clin. Nutr.* **21,** 1189 (1968).
2. W. H. CROSBY, in *Handbook of Physiology*. Alimentary Canal (Eds. W. HEIDEL and C. F. CODE), section 6, Vol. III, Ch. 78, p. 1553. Am. Physiol. Soc., Washington D.C. (1968).
3. B. CHENEY and C. A. FINCH, *Proc. Soc. exp. Biol. Med.* **103,** 37 (1960).
4. T. H. WILSON and G. WISEMAN, *J. Physiol.* **123,** 116 (1954).
5. H. A. KREBS and K. HENSELEIT, *Z. physiol. Chem.* **210,** 33 (1932).
6. R. D. BERLIN and R. A. HAWKINS, *Am. J. Physiol.* **215,** 932 (1968).
7. W. W. WESTERFIELD and D. A. RICHERT, *Proc. Soc. exp. Biol. Med.* **71,** 181 (1949).
8. D. W. WILSON and H. C. WILSON, *J. biol. Chem.* **237,** 1643 (1962).
9. P. F. HAHN, W. F. BALE, J. F. ROSS, W. M. BALFOUR and G. H. WHIPPLE, *J. exp. Med.* **78,** 169 (1943).
10. S. GRANICK, *Bull. N.Y. Acad. Med.* **25,** 403 (1949).
11. E. B. DOWDLE, D. SCHACHTER and H. SCHENKER, *Am. J. Physiol.* **198,** 609 (1960).
12. M. E. CONRAD, L. R. WEINTRAUB and W. H. CROSBY, *J. clin. Invest.* **43,** 963 (1964).
13. P. SALTMAN, *J. chem. Ed.* **42,** 682 (1965).
14. S. GREEN and A. MAZUR, *J. biol. Chem.* **227,** 653 (1957).