EFFECTS OF ALLOPURINOL ON IRON STORAGE IN THE RAT

BY

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Iron is stored in the liver and reticulo-endothelial system in the oxidized or ferric state and is bound to protein as ferritin or haemosiderin. Ferrous iron is less tightly bound to protein than is the ferric form. The mechanism of release of iron from ferritin is uncertain but there is some evidence that the xanthine oxidase system is of physiological importance in this step (Fig. 1). According to this hypothesis the reduction of ferric to ferrous ferritin occurs in association with the oxidation of xanthine and hypoxanthine to uric acid, xanthine oxidase acting as an electron donor in the reduction reaction and an electron acceptor in the oxidation reaction (Fridovich and Handler, 1958a, b).

Fig. 1.—Hypothetical role of xanthine oxidase in reduction of ferric to ferrous ferritin and oxidation of xanthine to uric acid (reproduced from *Lancet* (1966) 1, 239, by permission of the Editor).

The main evidence for a role of xanthine oxidase in the mobilization of storage iron comes from experimental observations. In a study of the mechanism of the reduction of ferritin iron, Green and Mazur (1957) found that ferritin was reduced by xanthine oxidase *in vitro* and small amounts of iron were liberated. Mazur, Green, Saha, and Carleton (1958) reported that, in dogs, the mobilization of storage iron induced by haemorrhagic shock was accompanied by a rise in serum uric acid (SUA) levels. They also demonstrated that the infusion of xanthine and hypoxanthine resulted in a rise, not only in SUA levels, but also in serum iron levels. Cheney and Finch (1960) showed that the parenteral administration of xanthine oxidase substrates in experimental animals was followed by a 50 to 100 per cent. rise in iron absorption. Mazur and Carleton (1965) demonstrated that the very high ferritin content of

the liver of the new born rat was associated with an absence of hepatic xanthine oxidase activity. Within 6 to 10 days after birth hepatic xanthine oxidase activity increased significantly coincident with a marked decrease in ferritin iron. This observation could be of considerable biological significance since it is immediately after birth that the need for iron for haemoglobin synthesis increases rapidly and the maternal source of iron is no longer present. Thus, with the appearance of xanthine oxidase in the liver, ferritin iron is released for transport to the bone marrow for incorporation into haemoglobin.

Unfortunately this hypothesis does not explain all the known or observed facts, and other experimental work has suggested that the relationship between xanthine oxidase and hepatic iron metabolism is an incidental one. For example, Strohmeyer, Greenberg, Moore, and Chalmers (1961) produced changes in iron metabolism in rats by hypoxia but they failed to demonstrate concomitant changes in hepatic xanthine oxidase activity. In a similar study, Strohmeyer, Miller, Scarlata, Moore, Greenberg, and Chalmers (1964) found that xanthine loading had no effect on the release of tissue iron or on iron absorption in rats. Kinney, Kaufman, and Klavins (1961) produced a reduction in hepatic xanthine oxidase activity with ethionine and sodium tungstate, but were unable to demonstrate any concomitant alteration in hepatic storage iron or iron absorption. Ayvazian (1964) reported a patient with both xanthinuria and haemochromatosis and a striking decrease in hepatic xanthine oxidase activity. He drew attention to the possible role of xanthine oxidase deficiency in idiopathic haemochromatosis, but a search of patients with haemochromatosis by ourselves and others has failed to reveal other similar cases or any abnormality in the SUA level. We also know of one patient with haemochromatosis and symptomatic gout (Price Evans, 1966)!

Because of the possible inverse relationship between hepatic xanthine oxidase activity and hepatic iron storage, we considered it important to watch for changes in iron metabolism following the therapeutic inhibition of xanthine oxidase. It is my task to outline the results of an experiment designed to study

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the effect of allopurinol on hepatic iron storage in rats, and Dr. Emmerson will then give the results of clinical studies.

Method

We had four groups of male Wistar rats aged 6 weeks. Each group was given one of the following diets and tap water *ad libitum*:

Group	No. of Rats	Diet
A	20	Laboratory meal
B	12	Laboratory meal + 5 per cent. ferric ammonium citrate
C	6	Laboratory meal + 0.01 per cent. allopurinol
D	6	Laboratory meal + 0.01 per cent. allopurinol + 5 per cent. ferric ammonium citrate

0.01 per cent. allopurinol is equivalent to approximately 10 mg./kg. body weight/day for each animal, and this is equivalent to about 300 mg./kg. for an adult human. All animals were kept under the same environmental conditions and were weighed weekly. Diets were replenished and weighed daily. The animals were killed at intervals and specimens of liver obtained for microscopy and chemical estimation of iron content.

Results

Fig. 2 shows the regression lines for hepatic iron concentration with time. There was a significant difference between the regression coefficients for rats receiving meal + allopurinol (Group C) and rats receiving laboratory meal alone (Group A). This

difference was significant at the 1 per cent. level of probability. The rise in hepatic iron concentration with time was steeper for rats receiving added allopurinol and iron (Group D) than for rats receiving added iron only (Group B), but the regression coefficients did not differ significantly. The presence of allopurinol did not affect food consumption or growth.

Fig. 3 shows the distribution and severity of the

Fig. 3.—Section of liver of rat treated with laboratory meal diet with added iron and allopurinol for 13 weeks. There is a heavy deposition of iron in a periportal distribution. Perls' stain. $\times 12$.

iron deposition in a histological section from the liver of a rat fed laboratory meal + iron + allopurinol (Group D) for 13 weeks. The accumulation of storage iron is seen in a periportal distribution. Comparable sections from rats fed meal + 5 per cent. ferric ammonium citrate (Group B) for 13 to 28 weeks showed lesser degrees of haemosiderosis, but there was considerable variation from one animal to another. The concentrations of iron in the livers of rats fed laboratory meal only and laboratory meal + allopurinol (Groups A and C) did not reach the levels necessary for histochemical staining (*i.e.* 0.2 to 0.25 mg./kg.).

Summary

Xanthine oxidase activity is associated under some experimental conditions with the release of storage iron from the liver. In this animal experiment a group of rats fed allopurinol had more iron in the liver than did control animals. It should be emphasized that the numbers of animals were small (only six rats in each allopurinol-treated group), but the results indicate a need for further investigation into the effect of xanthine oxidase inhibition on iron storage and in particular for careful observation of iron metabolism in patients receiving allopurinol.