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# MECHANISM OF RELEASE OF FERRITIN IRON *IN VIVO* BY XANTHINE OXIDASE<sup>1</sup>

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Studies of the biochemical mechanism for the release of iron from hepatic ferritin stores have revealed the participation of the enzyme xanthine oxidase (1). During this reaction between the enzyme and ferritin iron some of the iron in the ferric state is reduced to the ferrous form and can be removed from the protein in the presence of iron-binding agents such as  $\alpha,\alpha'$ -dipyridyl or the plasma iron-binding globulin, siderophilin. Evidence for the participation of this enzyme in the release of ferritin iron is based for the most part on *in vitro* studies using rat liver slices subjected to lowered oxygen tension. Such tissue slices accumulate ferrous-ferritin and high levels of hypoxanthine and xanthine, substrates for xanthine oxidase, as well as uric acid, their oxidation product. In addition, indirect evidence implicates this mechanism in the release of ferritin iron to the plasma in the intact animal; as a result of liver hypoxia following extensive blood loss, the plasma iron and uric acid of dogs is found to increase to a considerable extent. (2).

The results of the present study serve to confirm the participation of xanthine oxidase in the *in vivo* liberation of tissue iron to the circulation. Administration of xanthine oxidase substrates to a variety of animals serves to increase the activity of the enzyme in the tissues and brings about an increase in plasma iron. Studies of the reaction between xanthine oxidase and ferritin iron, as well as with other electron acceptors, serve to clarify the mechanism of this reaction.

## METHODS

Animals used in this study were anesthetized with 35 mg. per Kg. of sodium pentobarbital administered intravenously; when necessary, additional small doses of pentobarbital were given intramuscularly during the ex-

periment. In experiments with dogs and rabbits, blood samples (4 ml.) were withdrawn by means of a cannula placed in one of the femoral arteries. The cannula was filled with heparin solution and some of the heparin allowed to enter the circulation. This precaution serves to prevent occasional cloudiness of plasma samples. Blood samples were obtained from guinea pigs by means of a cannula in one of the carotid arteries. Prior to withdrawal of blood samples, the heparin-blood mixture in the cannula was removed and subsequently replaced.

Estimation of plasma uric acid was based on its reduction of the phosphomolybdic acid reagent (3). An additional plasma sample was incubated with the enzyme uricase to insure specificity of the uric acid determination (4). Plasma iron was determined by the method of Schade, Oyama, Reinhart and Miller (5) using a total of 1.0 ml. plasma; 0.5 ml. for the blank and 0.5 ml. for the terpyridyl reaction. Occasional confirmation of this method was carried out by duplicate analyses using the method of Kitzes, Elvehjem and Schuette (6) or Ramsay (7). The Schade method was preferred because of the small volume of plasma required and since small amounts of hemolysis do not interfere with the determination.

To study the effect of substrate concentration on the reduction by xanthine oxidase of molecular oxygen, reaction mixtures were prepared which contained the following: 1.5 ml. phosphate buffer, 0.1 M, pH 7.2; 0.3 ml. of a solution containing 1.2 mg. bovine serum albumin; 0.4 ml. 1:10 catalase (600 units); 0.4 ml. 1:50 xanthine oxidase (120 units); 1.0 ml. hypoxanthine of various concentrations, dissolved in 0.01 M NaOH; and water to make a total volume of 5.0 ml. The final pH was 7.4. The reaction mixture containing all ingredients except for hypoxanthine was incubated at 37° to achieve temperature equilibration and substrate was added at "zero" time. The mixtures were incubated in air for five minutes and the reaction stopped by the addition of 0.5 ml. each of 0.67 N H<sub>2</sub>SO<sub>4</sub> and 10 per cent sodium tungstate. Aliquots of the supernatant solution obtained after centrifugation were analyzed for uric acid.

For measurement of enzyme activity in the presence of methylene blue, all of the ingredients listed above together with 0.1 ml. of methylene blue solution containing 0.1 mg. of the dye were placed in the main compartment of a Thunberg tube. The substrate was placed in the stopper and the tubes alternately evacuated and filled with nitrogen four times. After temperature equilibration at 37° the contents of the tubes were mixed and the time required for complete decolorization noted.

<sup>1</sup> Aided by research grants from the Josiah Macy, Jr. Foundation, and the National Institutes of Health, United States Public Health Service (Grant A-1655).

Reduction of ferritin iron was measured in reaction mixtures which contained all of the ingredients listed above except that 0.8 ml. of horse spleen ferritin solution containing 12 mg. total iron was used instead of the dye and, in addition, 1.0 ml. of a 0.2 per cent aqueous solution of  $\alpha, \alpha'$ -dipyridyl. The reaction flasks containing all compounds except the substrate were incubated at 37° and flushed with tank nitrogen, and substrate was added at "zero" time. An equal volume of a cold mixture, containing two parts of 1 M acetate buffer, pH 4.6, and five parts of saturated ammonium sulfate, previously adjusted to pH 4.6, was added to stop the reaction. The mixtures were centrifuged to remove ferritin and the color due to the ferrous-dipyridyl complex read in a colorimeter against a standard ferrous iron solution treated in an identical manner. For each substrate concentration, two determinations were performed, one at 10 minutes and the other at 40 minutes. The reaction velocity was calculated for this 30 minute period.

Samples of purine were obtained through the courtesy of Drs. Fred Philips and Aaron Bendich of the Sloan-Kettering Institute and also from the Francis Earle Laboratories, Peekskill, N. Y. Other purines were obtained from Schwarz Laboratories, Mt. Vernon, N. Y.; catalase, uricase and xanthine oxidase from Worthington Laboratories, Freehold, N. J.

## RESULTS

### *Effect of hemorrhage or trauma on plasma uric acid*

The effect of hemorrhagic shock on plasma uric acid was studied inasmuch as dogs subjected to this procedure show an elevated level of plasma

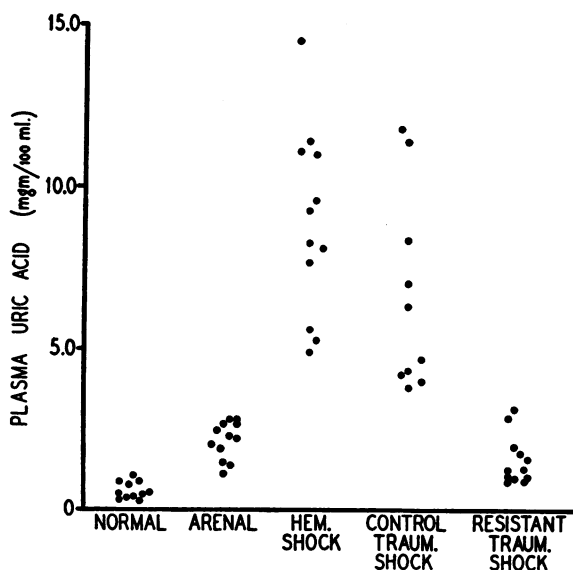


FIG. 1. EFFECT OF HEMORRHAGIC OR TRAUMATIC SHOCK ON RAT PLASMA URIC ACID

iron (2) and since our *in vitro* studies have shown a relationship between the enzyme xanthine oxidase and the release of ferritin iron (1). Hemorrhagic or traumatic shock results in an accumulation of products of nucleotide catabolism (8, 9). Therefore, determination of the end product of such catabolism, uric acid, would reveal the action of xanthine oxidase on hypoxanthine and xanthine resulting from such degradation products. Figure 1 shows uric acid concentrations in the plasma of rats subjected to hemorrhagic shock (10) and to traumatic shock (11) by tumbling in the Noble-Collip rotating drum.<sup>2</sup> Since the plasma uric acid of such animals is much higher than that of normal animals or of animals which had been nephrectomized, the increases which were observed were not due to cessation of kidney function in the shock state. Of interest are the results obtained with rats made resistant to the lethal outcome of traumatic shock, and subsequently subjected to the same intensity and duration of drumming as the nonresistant controls. Such animals, made resistant by repeated daily drummings of graded duration, after receiving the same number of turns as the controls, showed a much smaller increase of their plasma uric acid. The mechanism whereby such resistance develops is unknown.

### *Effect of intravenous purines on plasma iron*

In order to demonstrate that xanthine oxidase activity is directly concerned with the reduction and release of tissue ferritin iron in the intact animal, it was necessary to simulate one of the biochemical consequences of liver hypoxia, *i.e.*, elevated levels of xanthine oxidase substrates in the tissue. This may be accomplished by the intravenous administration of large quantities of such substrates in order to raise their concentration in tissues containing ferritin. Since hypoxanthine and xanthine are quite insoluble at neutral pH values, the quantity of these compounds which can be administered intravenously is limited. Hypoxanthine, in doses ranging from 10 to 42 mg. per Kg., was injected in a solution prepared by adding 4 ml. guinea pig plasma to 1 ml. of an alkaline solution of hypoxanthine followed by adjustment to pH 7.4 to 7.8 immediately prior

<sup>2</sup> The blood samples were kindly provided to us by Dr. S. Baez and Dr. Srikantia of the Department of Medicine, The New York Hospital.

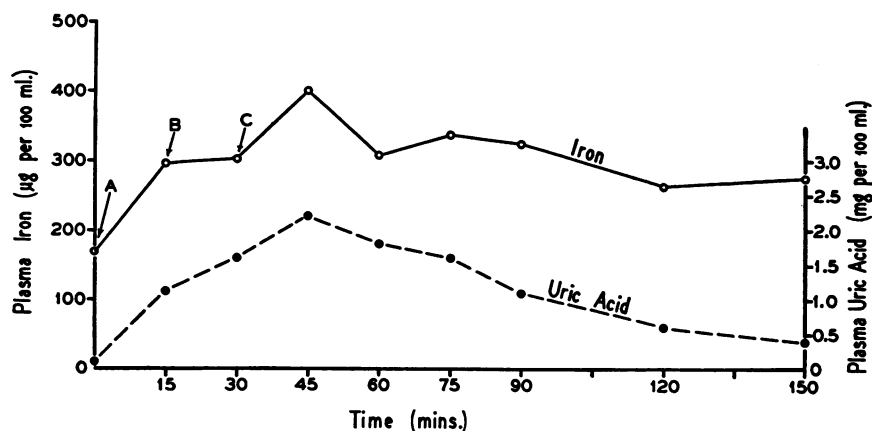


FIG. 2. EFFECT OF INTRAVENOUS HYPOXANTHINE ON DOG PLASMA IRON AND URIC ACID

Administered in three divided doses of 16.7 mg. per Kg. each at A (0 min.), B (15 min.) and C (30 min.).

to injection. When the higher doses of hypoxanthine were used, it is quite probable that some precipitated immediately following its injection into the circulation. Following the withdrawal of a control blood sample and the injection of hypoxanthine, blood samples were withdrawn at intervals of 15 and 30 minutes. In several guinea pigs, injected with an equal volume of normal plasma, the plasma iron values for these two time intervals were slightly lower than that of the pre-injection value. Table I gives the values for the maximum increase of plasma iron in guinea pigs and the duration of the increased level of plasma iron over that of the control. Because of the comparative insolubility of hypoxanthine, purine was used since it could be administered in larger quantities. Since this compound is oxidized by xanthine oxidase (12) it would be expected to

act in a manner similar to that of hypoxanthine. The results in Table I confirm this hypothesis. As proof of the *in vivo* conversion of purine to uric acid, plasma uric acid values following purine injections were elevated.

Since the number of blood samples which can be withdrawn from a guinea pig is limited, hypoxanthine or purine was administered to several dogs. Table I lists the increases of plasma iron and also demonstrates that the increased level of iron is maintained for periods greater than two hours in most animals. One dog was injected at intervals of 15 minutes with hypoxanthine, dissolved in dog plasma, in three divided doses totalling 50 mg. per Kg. In this animal the elevation of plasma iron was sustained over a period beyond two and one-half hours. Figure 2 shows the plasma iron and uric acid values in this dog

TABLE I  
Effect of purine and hypoxanthine on plasma iron in the dog and guinea pig

	Purine			Hypoxanthine		
	Dose	Plasma Fe increase	Return to normal	Dose	Plasma Fe increase	Return to normal
	mg./Kg.	µg./100 ml.	min.	mg./Kg.	µg./100 ml.	min.
Guinea pigs	100	127	> 30	10	55	> 30
	100	115	> 30	27	33	> 30
	150	145	> 30	39	66	> 30
				42	44	15
Dogs	100	53	>120	11.4	33	90
	100	96	>120	50*	236	>150
	200	196	>120			

\* Administered in three divided doses at intervals of 15 minutes.

TABLE II  
*Effect of purine and hypoxanthine on plasma iron in rabbits*

Purine			Hypoxanthine		
Dose	Plasma Fe increase	Return to normal	Dose	Plasma Fe increase	Return to normal
mg./Kg.	µg./100 ml.	min.	mg./Kg.	µg./100 ml.	min.
70*	107	>120	1.7	54	>120
80*	70	90	2.2	63	90
100*	33	>120	3.1	85	>120
100	44	>120	4.0	37	90
100	51	>120	4.9	83	>120
100	226	>120	6.2	28	30
100	110	>120	7.4	85	>120
200	37	60	9.1	100	>120
200	120	>120	10	57	>120
200	42	90	10	27	>60
200	44	90	10	17	90
200	50	60	10	17	60
200	52	120	12.5	50	60
200†	136	>120	15	17	60
200†	105	>120	15*	90	>120
250	26	>120	30*	20	>120
250	30	>120	50*	30	90
300	63	>120	50*	40	>120
300	60	>120	70*	30	90
300	134	>120	80*	23	60
300	155	>120			

\* Administered in three divided doses at intervals of 15 minutes.

† Splenectomized immediately before experiment.

at various time intervals. Elevated values of plasma uric acid accompanied the plasma iron rise.

Table II lists the results for 20 rabbits which received hypoxanthine dissolved in rabbit plasma in doses ranging from 1.7 to 80 mg. per Kg. and for 21 rabbits receiving purine in doses ranging from 70 to 300 mg. per Kg. In all cases increases in plasma iron were observed, although there was considerable variation in response to the injected material.

The specificity of the iron release "reaction" is seen from the results listed in Table III for a group of compounds other than hypoxanthine or purine. The administration of xanthine, inosine or adenylic acid resulted in a release of iron into the plasma. In all cases these compounds are either directly attacked by xanthine oxidase or are converted by enzymes in the animal to xanthine oxidase substrates. This was shown by the fact that their injection into the animal was followed by increased levels of uric acid in the plasma. On the other hand, uric acid itself, injected in amounts which provided levels as high as those achieved with purine or hypoxanthine, produced no increase in plasma iron. No hyperferremia was

produced by saline, glycine or sodium butyrate. Indeed, in all of the latter experiments, a fall in plasma iron was observed. This is undoubtedly due to the repeated removal of plasma with each blood sample and serves to make the hyperferremia obtained in previous experiments more significant.

TABLE III  
*Effect of various compounds on plasma iron of rabbits*

Compound injected	Dose	Plasma Fe increase	Return to normal
	mg./Kg.	µg./100 ml.	min.
Xanthine	50*	50	60
Adenylic acid	50	23	>120
Inosine	25.1	76	60
	45.6	15	90
	48.2	58	90
	48.2	55	>120
	98.5*	44	90
	150	23	150
Uric acid	50*	-36	
	62.5	-40	
Glycine	125	-20	
Butyric acid	163	-13	
Saline	(5 ml.)	-15	
	(5 ml.)	-17	
	(5 ml.)	-20	
	(5 ml.)	-22	
	(5 ml.)	-23	

\* Administered in three divided doses.

TABLE IV  
*Effect of hemorrhage and of purine on iron and uric acid of hepatic vein and femoral artery plasma*

	Time	Blood pressure	Plasma uric acid		Plasma iron	
			Hepatic vein	Femoral artery	Hepatic vein	Femoral artery
	<i>min.</i>	<i>mm. Hg</i>	<i>mg./100 ml.</i>		<i>µg./100 ml.</i>	
Hemorrhagic shock						
Dog A	0	120	0.3	0.2	78	78
	90	90	2.7	1.5		
	190	30	10.3	3.0	141	92
Dog B	0	150			95	95
	140	60			210	120
	245	32			435	330
Purine injection						
Dog C (100 mg./Kg.)	0		0.3	0.4	143	140
	60		4.3	3.4	370	336
	120		5.2	4.7	197	210
Dog D (200 mg./Kg.)	0		0.4	0.4	78	78
	15		3.0	2.2	140	102
	90		6.3	6.2	116	116

#### *Origin of iron release*

Experiments were designed to determine the site of release of iron into the plasma which results from hemorrhagic shock. Dogs were prepared with a catheter<sup>2</sup> placed by a surgical procedure directly into the hepatic vein which furnished blood samples arising from the liver and the portal circulation (spleen and small intestine). For comparison, blood samples were withdrawn simultaneously from the femoral artery to yield iron values for the mixed blood of the general circulation. The results (Table IV) show that the blood flowing from the liver contributes increased quantities of iron and uric acid to the general circulation since, at these time periods, the values for these constituents in the hepatic vein blood were higher than those in the arterial blood. Similar experiments<sup>3</sup> performed with dogs receiving a single dose of purine intravenously also showed differences in iron content between hepatic vein blood and that flowing from the femoral artery. These differences were smaller than those obtained in experiments using dogs in hemorrhagic shock. The relatively acute nature of the iron release phenomenon after a single injection of

purine as compared with the sustained liver hypoxia of hemorrhagic shock would make it difficult to obtain blood samples at the height of iron release after purine administration. That the spleen is not the exclusive site of iron liberation after purine administration can be concluded from several results listed in Table II, which show that splenectomized rabbits also respond to purine injection by a liberation of iron into the plasma.

#### *Relation of plasma iron to effect on blood pressure of nucleosides*

Since adenosine and inosine are reported to lower blood pressure after their administration (13), the possibility existed that the increase in plasma iron observed with inosine was due to tissue hypoxia caused by the lowered pressure rather than to its metabolic conversion to hypoxanthine. The injection of 150 mg. per Kg. of inosine to a rabbit resulted in an immediate drop in pressure from 120 mm. to 55 mm., which returned to normal in 15 minutes. Despite the brief period of lowered pressure the plasma iron reached a maximum at 60 minutes with an increase of 40 µg. iron per 100 ml. plasma. Adenosine, in a dose (19 mg. per Kg.) which did not result in any change in plasma iron, caused the blood pressure to fall from 110 mm. to 20 mm.

<sup>3</sup> We are indebted to Dr. George Wantz, Department of Surgery, The New York Hospital, for performing the catheterization.

TABLE V  
Effect of substrate concentration on reduction of xanthine oxidase acceptors

Hypoxanthine concentration $\mu\text{M/ml.}$	Enzyme activity in the presence of		
	Oxygen $\mu\text{M uric acid/hr.}$	Methylene blue $1/\text{t} \times 10^3 \text{ sec.}^*$	Ferritin iron $\mu\text{M Fe}^{++}/\text{hr.}$
0.05	3.2	21	0.2
0.10	3.8	21	2.1
0.20	3.4	14	3.2
0.50	1.4	9	3.2
1.00	0.8	7	3.0

\* Decolorization time.

immediately following the injection and to return to normal in 15 minutes.<sup>4</sup>

*Mechanism of reaction of ferritin iron with xanthine oxidase*

Xanthine oxidase is an unusual enzyme since it is capable of oxidizing a variety of substrates and also can reduce different acceptors. Among the latter are molecular oxygen, methylene blue, cytochrome C and, as we have demonstrated, ferritin iron. In light of the presence in the purified enzyme of flavin adenine dinucleotide (FAD), molybdenum and nonheme iron, experiments were performed in an attempt to determine the site on the enzyme which interacts with ferritin iron. Because of the well known inhibition of this enzyme, by increasing quantities of substrate in the presence of oxygen or methylene blue the effect of hypoxanthine concentration on the reduction of these acceptors was determined.

Table V lists the results obtained. It may be seen that the activity of xanthine oxidase is inhibited to a considerable extent beyond concentrations of 0.20  $\mu\text{M}$  hypoxanthine per ml. when molecular oxygen or methylene blue are used as acceptors, whereas no inhibition is obtained when ferritin iron is used.

We had previously reported (1) that the reduction of ferritin iron in the presence of oxygen was slightly greater than in its absence. In these

<sup>4</sup> Electrocardiogram recordings, taken by Dr. O. D. Kowlessar of the Department of Medicine, The New York Hospital, demonstrated that the heart rate fell to one-half its normal value at a time when the blood pressure was at its lowest, and returned to normal together with the blood pressure.

early experiments, an old preparation of enzyme had been used. For all of the experiments listed in Table V, a fresh preparation of the enzyme, used within several days of its receipt was utilized. When such a preparation was tested with ferritin in air and in nitrogen, the results shown in Table VI were obtained. The extent of ferritin iron reduction in nitrogen was much higher than in oxygen. On standing in the refrigerator, the loss of activity of the enzyme towards ferritin reduction in nitrogen was much greater than that measured in air, or than the activity of the enzyme as shown by uric acid formation in air. These results suggest that ferritin iron reduction in nitrogen involves a site on the enzyme different from that which is involved in the aerobic formation of uric acid or in ferritin reduction in air. Indeed, it appears possible that the reduction of ferritin iron in air may be partially due to uric acid which is formed.

DISCUSSION

Although the movement of iron from hepatic ferritin to the plasma in the animal has been postulated on the basis of studies with  $\text{Fe}^{59}$  (14), the appearance of increased quantities of iron in the plasma of animals injected with xanthine oxidase substrates or with compounds which can be converted to such substrates, serves to identify for the first time a specific biochemical mechanism which is concerned with the movement of iron in the body. That the hyperferremia resulting from purine administration is not confined to laboratory animals is suggested by the results obtained by Finch (15) after the intravenous injection of 1 Gm. of inosine into a man. The plasma iron rose from a control value of 72  $\mu\text{g.}$  per 100 ml. plasma to 103  $\mu\text{g.}$  per 100 ml. after

TABLE VI  
Effect of aging on xanthine oxidase activities \*

Age of enzyme	Uric acid formation in air	Ferritin iron reduction in	
		Air	Nitrogen
Fresh	100	6.4	20.7
3 weeks	100	5.4	10.7

\* The activity of the enzyme in terms of uric acid production ( $\mu\text{M}$  per hr.) is adjusted in each case to a value of 100, and the activity of the enzyme in terms of ferritin iron reduction ( $\mu\text{M Fe}^{++}$  per hr.) adjusted correspondingly.

5.5 hrs. and returned to preinjection values by 9.5 hrs. Our earlier *in vitro* studies showed that xanthine oxidase as well as uric acid could reduce ferritin iron. The results of the present study make it unlikely that uric acid is concerned with this reaction *in vivo*, since the injection of this compound does not lead to an increase of plasma iron, in contrast to the effect of xanthine oxidase substrates.

The fall in blood pressure which is observed with adenosine and inosine, although not directly related to the release of iron into the plasma, may be of some importance in circumstances occasioned by extensive hemorrhage or muscle injury. Evidence is available (8, 9) which shows a breakdown of nucleic acids and nucleotides and an increase in inorganic phosphate in tissues such as blood and liver in hemorrhagic shock and in blood and muscle in traumatic shock (16). A sustained release into the blood stream of such compounds would serve to maintain a low blood pressure for a relatively long period of time with a resultant hypoxia in tissues such as liver. A hypoxic liver would then release increased quantities of xanthine oxidase substrates and, consequently, increased quantities of iron to the plasma. In radiation injury (17) increases in plasma iron as well as evidence of increased destruction of nucleic acids, as shown by increased excretion of uric acid (18), have been reported.

Our findings also make it probable that the major source of iron liberated to the plasma as a result of hemorrhage or of purine administration is hepatic ferritin. Regulation of the flow of iron from the liver stores to the plasma can take place either by alteration of tissue levels of hypoxanthine and xanthine or their precursors, or of the level of the enzyme, xanthine oxidase. Lowered oxygen tension is one regulatory mechanism which increases the flow of hepatic iron to the plasma by increasing the level of tissue purines. Since hemorrhage produces tissue hypoxia, the result of such blood loss is a stimulation of iron release to the plasma upon which the bone marrow can now draw for accelerated hemoglobin synthesis. Similarly, one can explain the origin of the extra iron needed by the bone marrow for accelerated hemoglobin synthesis resulting from the polycythemia accompanying high altitude anoxia. It is suggested that the normal rate of

flow of iron from liver stores to the plasma is determined by low levels of xanthine oxidase substrates. The presence of specific inhibitors of xanthine oxidase, or the failure of the body to synthesize this enzyme (19), would serve to decrease the flow of iron from hepatic ferritin to the plasma. In addition to these factors, another regulatory factor, now under investigation, is the reincorporation of plasma iron into hepatic ferritin.

Fridovich and Handler<sup>5</sup> have postulated that enzyme-bound iron as well as its FAD are both concerned in the activity of xanthine oxidase (20). They picture the reaction of enzyme with hypoxanthine, in the presence of oxygen, as follows: reduction of an easily reducible FAD by interaction with substrate; transfer of an electron from the reduced FAD to enzyme  $Fe^{+++}$  forming  $Fe^{++}$ ; transfer of an electron from  $Fe^{++}$  to another FAD which reacts with oxygen. Since excess substrate inhibits the reaction of reduced enzyme with oxygen or methylene blue, the site of reaction of these acceptors must be different from that which reacts with ferritin iron. The binding of hypoxanthine by FAD makes it likely that oxygen and methylene blue normally react with FAD whereas ferritin iron reacts with enzyme-bound  $Fe^{++}$  resulting in the reduction of ferritin iron, thus bypassing the second FAD reaction. We have also tested the effect of hypoxanthine concentration on the ability of the enzyme to react with ferricytochrome C and have found no inhibition, as in the case of ferritin. This would suggest that cytochrome C also reacts with enzyme iron. However, the ability of our enzyme to reduce cytochrome C was much inferior to its reaction with oxygen, a finding reported by Westerfeld, Richert and Higgins (21), making an interpretation of the data difficult. Our results show that xanthine oxidase is capable of oxidizing substrates in the absence of oxygen and can also oxidize these substrates regardless of their concentration, provided ferritin iron is present. These conditions appear to be involved in physiological states characterized by liver hypoxia.

It is likely that the iron of ferritin in tissues other than the liver is reduced and released to

<sup>5</sup> We are indebted to Drs. I. Fridovich and P. Handler, Department of Biochemistry, Duke University, for a copy of their paper prior to its publication and for their suggestions.



the circulation by the xanthine oxidase mechanism. The spleen, small intestine during iron absorption (22), bone marrow and placenta (2) all contain ferritin. Xanthine oxidase is known to be present in the first two tissues but has not been investigated in the latter, although we have demonstrated the ability of rabbit bone marrow to reduce ferritin iron to the ferrous state (2). There is also evidence for the existence of hypoxic conditions in the normal placenta (23) during a time when increasing quantities of iron are transferred to the fetal circulation. The role of xanthine oxidase and its substrates in the release of iron from these tissues is under investigation.

#### SUMMARY

The release of ferritin iron in the liver to the plasma, after reduction by xanthine oxidase, has been confirmed in the intact animal for guinea pigs, rabbits and dogs. Administration of xanthine oxidase substrates or of nucleotides or nucleosides which can be converted to such substrates by the animal leads to an increase in plasma iron. Inhibition studies in the presence of excess substrate indicate that the reduction of ferritin iron by reduced xanthine oxidase involves a site on the enzyme different from that which reacts with molecular oxygen or methylene blue. It is suggested that the site of ferritin iron reduction is associated with the nonheme iron of the enzyme and takes place by direct electron transfer. The implications of this biochemical mechanism for the release of storage iron from the liver in various physiological states are discussed.

The physiological stimulus which is common to our experiments and which results in an accelerated release of iron from hepatic ferritin stores to the plasma is tissue hypoxia. This produces an increase in level of xanthine oxidase substrates and a consequent reduction and release of ferritin iron to the plasma. Since the result of lowered oxygen tension is a stimulation of erythrocyte synthesis, and therefore of iron incorporation into hemoglobin, the ferritin-xanthine oxidase system constitutes part of a homeostatic mechanism for the regulation of the level of iron in the circulation.

#### REFERENCES

1. Green, S., and Mazur, A. Relation of uric acid metabolism to release of iron from hepatic ferritin. *J. biol. Chem.* 1957, **227**, 653.
2. Mazur, A., Baez, S., and Shorr, E. The mechanism of iron release from ferritin as related to its biological properties. *J. biol. Chem.* 1955, **213**, 147.
3. Folin, O. Standardized methods for the determination of uric acid in unclaked blood and in urine. *J. biol. Chem.* 1933, **101**, 111.
4. Blauch, M. B., and Koch, F. C. A new method for the determination of uric acid in blood, with uricase. *J. biol. Chem.* 1939, **130**, 443.
5. Schade, A. L., Oyama, J., Reinhart, R. W., and Miller, J. R. Bound iron and unsaturated iron-binding capacity of serum. Rapid and reliable quantitative determination. *Proc. Soc. exp. Biol. (N. Y.)* 1954, **87**, 443.
6. Kitzes, G., Elvehjem, C. A., and Schuette, H. A. The determination of blood plasma iron. *J. biol. Chem.* 1944, **155**, 653.
7. Ramsay, W. N. M. The determination of iron in blood plasma or serum. *Biochem. J.* 1953, **53**, 227.
8. McShan, W. H., Potter, V. R., Goldman, A., Shipley, E. G., and Meyer, R. K. Biological energy transformations during shock as shown by blood chemistry. *Amer. J. Physiol.* 1945, **145**, 93.
9. Le Page, G. A. The effects of hemorrhage on tissue metabolites. *Amer. J. Physiol.* 1946, **147**, 446.
10. Baez, S., Zweifach, B. W., Pellon, R., and Shorr, E. A renal factor in vascular response to hemorrhagic and traumatic shock. *Amer. J. physiol.* 1951, **166**, 658.
11. Noble, R. L., and Collip, J. B. A quantitative method for the production of experimental traumatic shock without haemorrhage in unanaesthetized animals. *Quart. J. exp. physiol.* 1941-2, **31**, 187.
12. Krebs, H. A., and Örström, A. Microdetermination of hypoxanthine and xanthine. *Biochem. J.* 1939, **33**, 984.
13. Drury, A. N. The physiological activity of nucleic acid and its derivatives. *Physiol. Rev.* 1936, **16**, 292.
14. Jensen, W. N., Bush, J. A., Ashenbrucker, H., Cartwright, G. E., and Wintrobe, M. M. The kinetics of iron metabolism in normal growing swine. *J. exp. Med.* 1956, **103**, 145.
15. Finch, C. A. Personal communication.
16. Bielschowsky, M., and Green, H. N. Shock-producing factors from striated muscle. II. Fractionation, chemical properties and effective doses. *Lancet* 1943, **245**, 153.
17. Chanutin, A., and Ludewig, S. Effect of whole body X-irradiation on serum iron concentration of rats. *Amer. J. Physiol.* 1951, **166**, 380.

18. Jackson, K. L., and Entenman, C. Urinary excretion of uric acid and allantoin by the X-irradiated adrenalectomized rat. *Proc. Soc. exp. Biol. (N. Y.)* 1958, **97**, 184.
19. Westerfeld, W. W., and Richert, D. A. Liver and intestinal xanthine oxidases in relation to diet. *J. biol. Chem.* 1951, **192**, 35.
20. Fridovich, I., and Handler, P. Xanthine oxidase. II. Studies of the active site. *J. biol. Chem.* 1958, **231**, 899.
21. Westerfeld, W. W., Richert, D. A., and Higgins, E. S. Functional groups in xanthine oxidase *in* Inorganic Nitrogen Metabolism, W. D. McElroy, and B. Glass Eds. Baltimore, The Johns Hopkins Press, 1956, p. 492.
22. Granick, S. Ferritin. IX. Increase of the protein apoferritin in the gastrointestinal mucosa as a direct response to iron feeding. The function of ferritin in the regulation of iron absorption. *J. biol. Chem.* 1946, **164**, 737.
23. Walker, J., and Turnbull, E. P. N. Haemoglobin and red cells in the human foetus. *Lancet* 1953, **265**, 312.