Xanthine Oxidase Activity and Iron Storage in the Liver.*† (26833)

THOMAS D. KINNEY, NATHAN KAUFMAN AND JANIS V. KLAVINS
Department of Pathology, Duke University Medical Center, Durham, N.C.

It has been shown by others that xanthine oxidase participates in the release of iron from hepatic ferritin. The initial studies were carried out *in vitro* on rat liver slices subjected to lowered oxygen tension(1). With increasing activity of xanthine oxidase there was an increase in release of iron. Subsequent studies in intact animals supported this view(2). These authors suggested that ferritin iron was reduced to a more labile ferrous state making the iron available to iron binding agents such as transferrin. This then is reflected in the elevated plasma iron level.

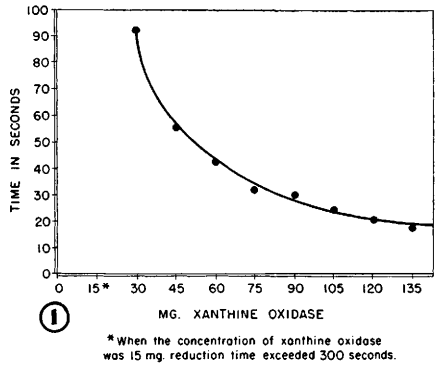
If release of ferritin iron depends on xanthine oxidase activity it might be expected that decreasing the activity of this enzyme should result in accumulation of iron in the tissues. In the experiments to be described, liver xanthine oxidase activity was lowered by feeding sodium tungstate but there was no concomitant increase in liver iron stores.

Experimental procedure. Male albino rats of the Sprague Dawley strain, each weighing approximately 100 g, were fed a basic synthetic diet of the following composition: 65.7% glucose, 18% casein, 4% salt mixture(3), 11% corn oil, 0.3% choline chloride, and 1% iron citrate. To this diet were added crystalline vitamins in the following amounts per 100 g of diet: thiamine chloride 400 μg, pyridoxine hydrochloride 400 μg, riboflavin 800 μg, calcium pantothenate 1.5 mg, and nicotinic acid 2.5 mg. Each 100 g of diet was supplemented with approximately 60 U.S.P. units of Vit. A and 1 U.S.P. unit of Vit. D (Haliver oil, Parke-Davis). All animals were housed individually and given water *ad libitum*. The animals were divided into 2 groups of 16 each. In the experimental group the animals were fed the basic diet *ad libitum* supplemented with 16.84 mg of sodium tungstate ($Na_2WO_4 \cdot 2H_2O$) per 100 g of diet. The other group containing the pair fed control animals was fed the basal diet without sodium tungstate supplement. All rats were weighed twice a week. Hemoglobin and hematocrit determinations were performed at beginning and at termination of experiments. After 4 weeks the animals were sacrificed. The entire carcass was utilized for iron analysis in one half of each group. In these animals the intestines were removed, washed, and analyzed separately. The remainder of the animals in each group were autopsied and sections taken from liver, duodenum, pancreas, and pyloric portion of the stomach for histologic examination. Sections were stained with hematoxylin-eosin and for iron, using Perls' method. Livers were weighed and, after sections were taken for histologic examination, were analyzed for iron. Iron analyses were performed by the method of Kitzes, Elvehjem, and Schuette(4).

Liver xanthine oxidase was determined by the method of Remy, *et al.*(5) modified slightly by utilizing xanthine instead of hypoxanthine for the substrate. A 10% wt/vol suspension of each liver sample was prepared in 0.04 M phosphate buffer. For determination of enzyme activity 0.2 cc of the tissue extract was used. This represented 20 mg of the original liver sample. Enzyme activity was expressed as number of seconds required by the enzyme extracted from each 20 mg of liver to reduce the dye (Sodium 2,6-dichlorobenzenoneindo-3′-chlorophenol) from a colorimeter reading of 100 to one of 80. A Klett-Summerson colorimeter with a 660 mμ filter was used. In addition, a known amount of commercially available xanthine oxidase (Nutritional Biochemical Corp.) was tested by this method. Relationships between reduction time and enzyme concentration are shown in Fig. 1. When concentration of xan-

* This work was supported by U. S. Public Health Service grant.

† Presented in part at 45th Annual Meeting of Am. Soc. Exp. Path., April, 1961.



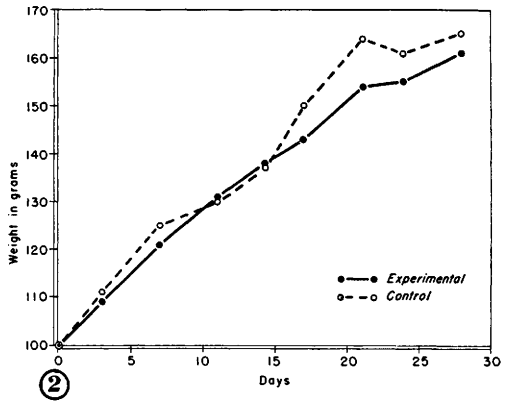


FIG. 1. Relationships between reduction time and xanthine oxidase concentration.
FIG. 2. Avg weight changes in rats on a sodium tungstate supplement.

thine oxidase was 15 mg reduction time exceeded 300 seconds.

Results. The time required for dye reduction in the experimental group receiving the sodium tungstate was greater than 300 seconds per 20 mg liver (Table I). In comparison the time required for similar reduction in the pair fed control group ranged from 38 to 162 seconds with an average of 76 seconds. This indicated marked reduction in xanthine oxidase activity in the experimental group on the sodium tungstate supplement.

Liver iron values ranged from 0.98 to 1.83 mg per liver with an average of 1.25 mg in experimental animals and 0.85 to 1.49 mg with an average of 1.25 mg of iron per liver in pair fed control animals. These values were not statistically different when the 2 groups were compared ($p>0.1$). Further, when iron values were calculated as mg per 100 g of liver there was no significant variation. In addition, no differences were noted in amount and distribution of liver iron as determined histochemically. Occasionally small amounts of iron were seen in Kupffer cells and parenchymal cells in both experimental and control animals. When the entire carcasses were analyzed, experimental animals contained an average of 7.34 mg (6.40 to 8.52) and pair fed control animals contained an average of 7.65 (6.04 to 9.16) mg of iron per animal. As with the liver iron values, these values were not significantly different ($p>0.1$). There were no statistically significant differences in hemoglobin and hematocrit values when experimentals and controls were compared (Table I).

There was no significant difference in weight gain between experimental and the control animals (Fig. 2). Histologic changes were not seen in liver, pancreas, duodenum, or pyloric end of the stomach.

Discussion. It has been suggested that xanthine oxidase facilitates release of iron from liver *in vitro* by reducing the ferritin bound iron to the more readily dissociable ferrous states(1). If this enzyme is physiologically important in this role then the ability of the tissue to mobilize iron from its storage form would depend upon the level of activity of xanthine oxidase and a decrease of xanthine oxidase would be expected to give rise to an accumulation of iron in liver cells.

Our previous experiments showed that dietary ethionine decreased liver xanthine oxidase activity and increased iron deposition (6). Analysis of the covariates, total liver xanthine oxidase activity and total liver iron, showed a significant negative regression of liver iron values upon xanthine oxidase activity. This inverse relationship suggested

TABLE I. Effect of Tungstate Administration.

	Exp. group	Controls
Xanthine oxidase activity, sec /20 mg liver	>300	76
Total liver iron, mg	1.25	1.25
Liver iron, mg/100 g liver	21.9	22.4
Carcass iron, mg	7.34	7.65
Hemoglobin, g %	15.4	14.4
Hematocrit, %	51	47

that either the increase in liver iron values was caused by changes in xanthine oxidase or that both the changes in liver iron and xanthine oxidase activity were an expression of some other unidentified cause. In addition, statistical analysis indicated that the changes in xanthine oxidase could not have accounted for all the variation in liver iron values, and cast doubt on the hypothesis of a causal relationship between xanthine oxidase activity and iron deposition in liver *in vivo*.

To avoid the many effects of ethionine and still produce a lowering of xanthine oxidase activity, the present experiments were set up utilizing sodium tungstate(7) as a means of depressing xanthine oxidase activity. Under these circumstances there was a definite decrease in xanthine oxidase activity but no histologic changes in the animals. Neither was there any difference in weight gain. When total carcass iron values were compared, the animals with low liver xanthine oxidase activity had the same amount of iron in the body as the pair fed control animals without the lowered xanthine oxidase activity. This indicated that there was no difference in absorption of iron in both groups. Neither was there any alteration in storage of iron in the liver (Table I). In addition, there was no lowering of hemoglobin values in the experimental animals, indicating that they did not suffer from anemia as a result of possible decrease in release of body storage iron. It was concluded from these studies that the lowering of liver xanthine oxidase activity did not affect iron deposition in the liver or iron absorption. Recently Strohmeyer, *et al.*(8) utilizing a different approach failed to demonstrate a relationship between liver xanthine oxidase activity and iron metabolism. It would seem from our study that whatever role the liver xanthine oxidase may play in iron metabolism it has no direct effect on liver iron storage or on iron absorption.

Summary. Sodium tungstate was added to the diet to decrease liver xanthine oxidase activity. There were no concomitant changes in liver iron or iron absorption associated with the decrease in xanthine oxidase activity.

1. Green, S., Mazur, A., *J. Biol. Chem.*, 1957, v227, 653.

2. Mazur, A., Green, S., Saha, A., Carleton, A., *J. Clin. Invest.*, 1958, v37, 1809.

3. Hegsted, D. M., Mills, R. C., Elvehjem, C. A., Hart, E. B., *J. Biol. Chem.*, 1941, v138, 459.

4. Kitzes, G., Elvehjem, C. A., Schuette, H. A., *ibid.*, 1944, v155, 653.

5. Remy, C. N., Richert, D. A., Doisy, R. J., Wells, I. C., Westerfeld, W. W., *ibid.*, 1955, v217, 293.

6. de Monterice, B., Kaufman, N., Kinney, T. D., Klavins, J. V., *Arch. Path.*, in press.

7. Higgins, E. S., Richert, D. A., Westerfeld, W. W., *J. Nutr.*, 1956, v59, 539.

8. Strohmeyer, G. W., Greenberg, M. S., Moore, E. W., Chalmers, T. C., *Clinical Research*, 1961, v9, 167.

Received May 15, 1961. P.S.E.B.M., 1961, v108.

Changes in Total Peripheral Resistance in Endotoxin Shock.* (26834)

LERNER B. HINSHAW,† JAMES A. VICK, LORENTZ E. WITTMERS, DAVID M. WORTHEN, DAVID L. NELSON AND ORVILLE P. SWENSON

Department of Physiology, University of Minnesota Medical School, Minneapolis

Previous reports have described changes in total peripheral resistance during irreversible hypotension following injection of endotoxin(1-5). Results, however, have differed and the question of resistance changes in endotoxin shock is not settled. Two general procedures have been previously employed for calculation of vascular resistance: (a) measurements of arterial pressures during constant cardiac inflow in perfused animals (3,4), and (b) measurements of arterial pres-

* This research was supported by grants from U. S. Public Health Service and Minnesota Heart Assn.

† Research was performed during term of Lederle Medical Faculty Award, 1959-1962.