XANTHINE OXIDASE ACTIVITY IN REGENERATING LIVER*

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ABSTRACT

The xanthine oxidase activity of mouse regenerating liver has been shown to be elevated during the period of rapid liver growth and proliferation. This increase is evident when the enzyme activity is expressed per unit wet tissue weight, per unit nitrogen, or per cell. The adrenal cortex probably plays only a minor role in implementing this phenomenon. Further augmentation of the xanthine oxidase level of regenerating liver is not induced by the administration of large quantities of the substrate, xanthine, to the animal.

INTRODUCTION

In the course of a study on xanthine-induced adaptive enzyme formation in mammalian tissues (10), it seemed profitable to investigate the possibility that the proliferating cells of regenerating liver, when "reared" in an environment rich in exogenously supplied xanthine, might more readily form increased amounts of xanthine oxidase adaptively then would the older more stable population of cells present in resting liver. An experiment designed to test this hypothesis revealed that mouse regenerating liver manifests no induction of xanthine oxidase following xanthine administration, but, rather, that liver regeneration *per se* is attended by a marked elevation in xanthine oxidase level as compared with normal liver, with no further increase manifested upon parenteral xanthine administration.

Although there is much evidence indicating that active synthetic mechanisms are operative during liver regeneration (4, 9, 19, 21, 26), the literature reveals a dearth of examples of elevations in the concentrations of enzymes during regeneration. Greenstein, in fact, stresses the enzymatic similarity between normal and regenerating liver, in contrast to that between neoplastic and fetal liver (12). Among the many enzymes studied in regenerating liver, only the alkaline phosphatases (20, 25) and adenosine deaminase (24) were clearly shown to be present at concentrations greater than normal and to be directly correlated with the active growth phase of the regeneration.

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In light of these factors, it seemed of some import to define precisely the pattern of xanthine oxidase levels during liver regeneration in the mouse.

METHODS

Male C57 Bl/6 JAX mice of body weights ranging between 21 and 34 gm. were employed in these experiments. All animals were fed Purina chow and were allowed tap water *ad libitum*.

The following experimental plans were adopted. In the adaptive enzyme experiment groups of six mice were partially hepatectomized on 12 successive days and groups of four mice were reserved as unoperated controls. During the postoperative period all animals were allowed a choice of 20 per cent glucose or tap water. The hepatectomized mice were given a single intraperitoneal injection of 0.5 ml. 20 per cent glucose 10 hours after surgery. Half of each group of mice were given intraperitoneally 0.12 ml. of 0.85 per cent NaCl per 10 gm. body weight; the other half 0.12 ml. of 2 per cent xanthine suspension in saline per 10 gm. body weight. Each mouse was given a total of eleven injections: two doses on the day of surgery and on each of the first 4 days following hepatectomy; one dose on the 5th posthepatectomy day. Four hours later the animals were sacrificed, the livers were removed, one to three livers within each group were pooled, and xanthine oxidase determinations were performed on homogenates prepared from the pooled livers.

In the study designed to follow the pattern of xanthine oxidase levels during liver regeneration, approximately twelve mice were hepatectomized on successive days and two to four mice were laparotomized. A group of untreated stock mice was reserved as unoperated controls and maintained under conditions identical with those of the mice submitted to surgery. During the postoperative period all animals were given a choice of 5 per cent glucose or tap water. No further treatment was administered to these animals. At the designated intervals following surgery, the mice were sacrificed and their livers removed. One to four livers were pooled within each group, homogenates were prepared, nuclear counts were made, and the xanthine oxidase activity and total nitrogen were determined on each homogenate. The adrenals were removed from each mouse and weighed.

The partial hepatectomies were performed employing a modification of the procedure of Higgins and Anderson (15). It was found that a single ligation, around the pedicle of the left lateral lobe and the two portions of the medial lobe as prescribed by Higgins and Anderson for the rat resulted in high mortality in the mouse, due possibly to the removal of the gall bladder and disruption of the biliary system. Therefore the following modifications of the Higgins and Anderson procedure were undertaken: the ventral hepatic mesentery was severed; two ligations were made, one around the pedicles of the left lateral lobe and the left portion of the median lobe (left central lobe), and another around the pedicle of the right portion of the median lobe (right central lobe). Care was taken to leave the gall bladder and biliary ducts intact. After each ligation was made, the hepatic tissue distal to the ligature was cut free as close to the ligature as possible. Laparotomies were performed in the following manner: a medial incision into the body wall was made; the ventral hepatic mesentery was sectioned; the hepatic lobes analogous to those removed in partial hepatectomy

were manipulated. In both the hepatectomies and laparotomies the body wall and skin were sutured in two layers.

One to four livers pooled from animals receiving identical treatment were homogenized for 2 minutes in a Teflon homogenizer. The homogenization procedure and the xanthine oxidase determinations were carried out in the manner described by Axelrod and Elvehjem (1). A modification of the procedure of Bass *et al.* (2) was adopted for counting nuclei in duplicate samples of the same homogenates which were utilized for the enzyme assay. Twenty mg. per cent crystal violet in 6 per cent acetic acid served as the diluent. Nuclei in five red cell squares of each chamber of a hemocytometer were counted for each duplicate sample. The maximum deviation from the mean of the four counts thus obtained for each homogenate rarely exceeded

TABLE I

The Effect of Substrate Administration on Xanthine Oxidase Activity in Normal and Regenerating Livers

Animal	Solution injected	No. of homogenates	Xanthine oxidase activity	
			O_2 uptake	Increase over intact saline control
			µl. per gm. wet liver	*per cent*
Intact	Saline	7 (13)*	364 ± 34‡	—
Intact	Xanthine	7 (14)	427 ± 60	17.3
Hepatectomized	Saline	11 (19)	558 ± 32	53.3
Hepatectomized	Xanthine	10 (17)	518 ± 32	42.3

* The number in parenthesis represents the actual number of animals used. Livers from these animals were so pooled as to comprise the number of homogenates indicated.

‡ Mean ± standard error (S.E.)

S.E. = $\sqrt{x^2/n(n\text{-}1)}$

plus or minus 10 per cent. Total nitrogen estimations were made using the micro-Kjeldahl method (14).

RESULTS

The initial observation on xanthine oxidase levels in regenerating liver was made during an experiment designed to determine whether regenerating liver possesses a greater ability to form xanthine oxidase adaptively in response to parenteral administration of xanthine than does resting liver. Table I summarizes the xanthine oxidase levels of resting liver and of 5 posthepatectomy day old regenerating liver. It is clear that regenerating liver possesses over 50 per cent more xanthine oxidase activity than does resting liver. Since the p value equals 0.01,[1] this difference is statistically significant. Xanthine suspension injected during the course of 5 days produces a 17 per cent increase in the xan-

[1] The probability values (p) are obtained by submitting the data to the t test (23). Probability values of 0.01 or less are considered statistically significant.

thine oxidase of intact normal livers; this increase is statistically insignificant ($p = 0.3$). The high levels of xanthine oxidase of regenerating liver are increased no further with parenteral administration of xanthine.

A kinetic study was undertaken to determine the pattern of xanthine oxidase elevation during the course of regeneration. Liver xanthine oxidase was determined on groups of regenerating livers at various intervals during 22 days following partial hepatectomy. These values are compared to resting livers from mice laparotomized on the same days. To ascertain the possible influence of surgical stress on xanthine oxidase, enzyme assays of livers from a group of

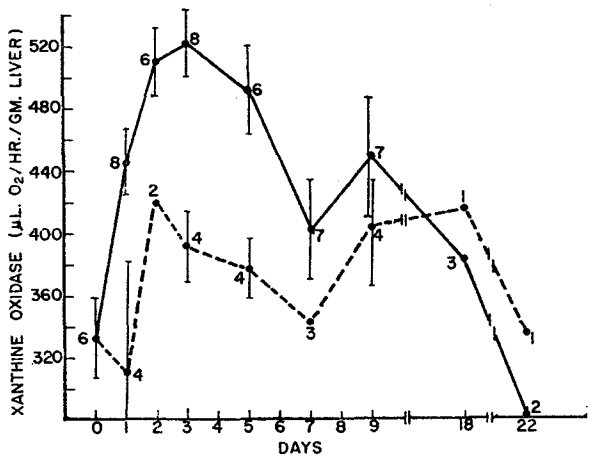

FIG. 1. Xanthine oxidase activity per gram wet liver tissue following partial hepatectomy and laparotomy. The solid line represents regenerating liver; the broken line, livers from laparotomized mice. The vertical bars denote the standard error of the mean. The numbers at each point refer to the number of homogenates assayed. The value at 0 days represents unoperated controls.

unoperated controls are also reported. Fig. 1 depicts the liver xanthine oxidase activity per gram wet liver weight as a function of time following partial hepatectomy. Statistically significant elevations of xanthine oxidase activity of 22, 33, and 30 per cent are found in regenerating livers 2, 3, and 5 days respectively after partial hepatectomy, as compared with their laparotomized controls (in each case $p < 0.01$). The maximum elevation, achieved at 3 days, is followed by a slow decline; approximately 2 weeks posthepatectomy the xanthine oxidase levels of the hepatectomized animals return to the laparotomized control levels. The means of the xanthine oxidase levels of the laparotomized mouse livers are in general higher than the mean value for the unoperated control livers, indicated at day zero. These elevations, however, are statistically insignificant.

Since liver regeneration is accompanied by shifting patterns of lipide concentration and of hydration (11, 26), it seemed essential to express the enzyme

activities on a basis which is independent of these variables. For this reason, the total nitrogen content of the liver was determined. The results recorded in Fig. 2 show the nitrogen content per gram regenerating and laparotomized livers at various intervals following surgery as compared with unoperated controls. It can be seen that in early regenerative stages the nitrogen content of the liver is considerably depressed (a statistically significant depression of 22 per cent at 1 day ($p < 0.01$)) and then climbs continuously until complete restoration of nitrogen is achieved at 5 days. The surgical stress of laparotomy produces no significant change in the nitrogen content of resting livers.

A replot of the xanthine oxidase activity on a per nitrogen basis ($Q^N_{O_2}$), is presented in Fig. 3. Xanthine oxidase activities expressed as $Q^N_{O_2}$ show a more

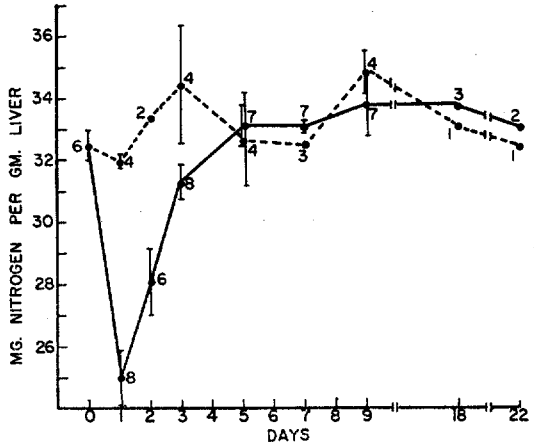

FIG. 2. Total nitrogen per gram wet liver tissue following hepatectomy and laparotomy. Details are as in Fig. 1.

striking elevation than when expressed on a wet tissue weight basis, *viz.*, statistically significant posthepatectomy enzyme elevations of 71, 45, 46, and 31 per cent for 1, 2, 3, and 5 days respectively over the corresponding laparotomized groups (each case $p < 0.01$). The complete restoration of liver xanthine oxidase activity per unit nitrogen to normal levels is again achieved at about 2 weeks. It can thus be seen that despite a lowered total tissue nitrogen concentration, in the early stages, xanthine oxidase is present at an elevated level.

Price and Laird (21), Einhorn *et al.* (8), and others have stressed the importance of expressing the amounts of cellular constituents of rapidly proliferating tissues, such as regenerating liver, on a per cell basis if knowledge of the alterations in any one constituent of the average cell is desired. Variations in volume, lipide, water, and nitrogen composition of the cells during the course of regeneration can be accounted for by expressing xanthine oxidase activity on a per cell basis. Fig. 4 shows the number of nuclei per gram wet liver tissue of regen-

erating and resting liver during the 3 weeks following hepatectomy and laparotomy respectively. It is evident that a given wet weight of regenerating liver contains fewer cells than does resting liver from laparotomized mice; cell counts on regenerating liver range between 16 and 29 per cent below those of livers

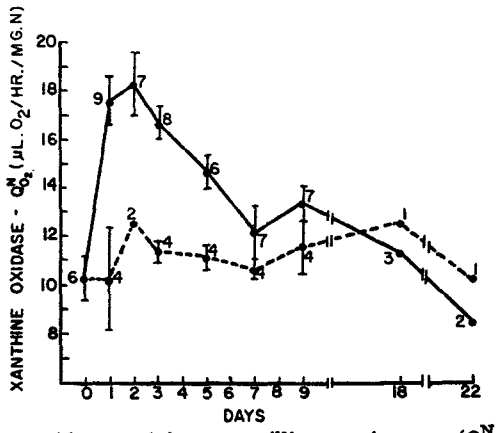

FIG. 3. Xanthine oxidase activity per milligram nitrogen ($Q^N_{O_2}$) following hepatectomy and laparotomy. Details are as in Fig. 1.

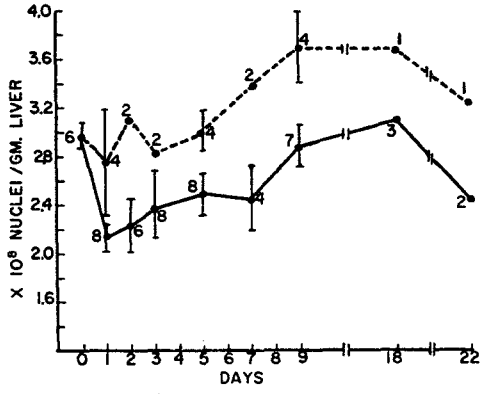

FIG. 4. Number of nuclei per gram wet liver tissue following hepatectomy and laparotomy. Details are as in Fig. 1.

from laparotomized mice. Restoration of cellularity to unoperated control values is slowly achieved during 22 days after hepatectomy, although restoration to laparotomy values is not completely achieved during this period.

Xanthine oxidase activity replotted on a per cell basis may be seen in Fig. 5. Since the cellularity of actively regenerating liver is lower than that of intact liver (Fig. 4), it follows that increased elevations in enzyme activity per unit cell of regenerating livers are even more striking and of greater statistical

significance than when expressed on a wet liver weight basis. Increases of 64, 69, 67, 74, 50, and 45 per cent occur at 1, 2, 3, 5, 7, and 9 days respectively after

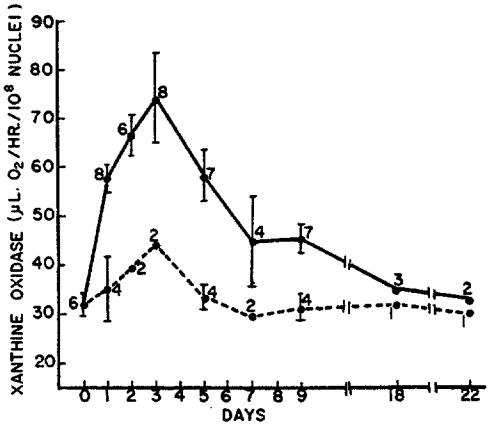

FIG. 5. Xanthine oxidase activity per 10^8 nuclei following hepatectomy and laparotomy. Details are as in Fig. 1.

TABLE II

Mouse Adrenal Weights after Hepatectomy and Laparotomy

Time after surgery	Adrenal weights (mg./gm. body weight)			Laparotomized / Unoperated controls × 100
	Hepatectomized	Laparotomized	Hepatectomized / Laparotomized × 100	
days				
1	16.4 ± 0.5* (17)‡	13.6 ± 0.8 (5)	121	107
2	15.1 ± 0.8 (13)	14.0 ± 1.2 (4)	108	110
3	14.2 ± 0.6 (13)	12.1 ± 0.6 (5)	117	95
5	14.3 ± 0.5 (13)	13.7 ± 0.7 (7)	104	108
7	14.4 ± 0.7 (10)	14.6 ± 1.2 (4)	99	115
9	14.3 ± 0.6 (13)	12.3 ± 0.7 (7)	116	97
17–18	17.6 ± 1.7 (4)	18.6 (1)	95	146
22	11.7 ± 0.3 (4)	10.9 (1)	107	86
Unoperated controls.....	12.7 ± 0.7 (11)			

* Mean ± S.E.

‡ The number in parenthesis represents the number of animals used.

surgery (in each case $p < 0.01$). Normal xanthine oxidase levels in regenerating livers are attained, again, at about 2 weeks. These data therefore indicate that a given weight of regenerating liver during its early stages possesses approximately 20 per cent fewer cells than does intact laparotomized liver, and that

each cell contains approximately 70 per cent more xanthine oxidase than do cells from intact livers.

Since cortisone has been shown to elevate liver xanthine oxidase (5), it seemed important to assess the role played by the adrenal in implementing the observed xanthine oxidase elevations. Although laparotomy itself serves as a control for a part of the surgical trauma, the extensive sectioning involved in removal of 60 per cent of the liver, as well as the vast physiological upheaval resulting from such an ablation, adds considerably to the total trauma of the hepatectomized mice as compared with those that were laparotomized. If the trauma induced by partial hepatectomy serves as a persistent non-specific stimulus for adrenal cortical secretion, adrenal hypertrophy should result (22). Table II, therefore, presents the weights of pairs of adrenals from individual animals, expressed per unit body weight. A small adrenal enlargement can be seen in the hepatectomized mice compared to their laparotomized controls; this hypertrophy, however, is inconsistent. (p Values are < 0.01, > 0.40, 0.01 to 0.02, > 0.50, 0.02 to 0.05 for 1, 2, 3, 5, and 9 days respectively after hepatectomy). Whether this minor adrenal hypertrophy could be responsible for the marked xanthine oxidase elevations obtained during the first 5 days of regeneration remains uncertain. Adrenal weights of laparotomized mice are not significantly different from those of the unoperated controls at any time interval.

DISCUSSION

Regenerating liver is clearly not a suitable system for either the true induction of xanthine oxidase by its substrate, nor the selection of cells rich in xanthine oxidase, despite the fact that the liver cells are proliferating and developing in a xanthine-rich milieu. However, the fact that regenerating liver itself is shown to possess a high xanthine oxidase concentration may preclude any further elevations by exogenous inductors.

In this study considerable elevations in xanthine oxidase levels of mouse regenerating liver are evidenced, whether the xanthine oxidase activity is expressed per unit wet tissue weight, per unit nitrogen, or per cell. This finding differs from that of Greenstein (13), who reported the xanthine oxidase activity in rat regenerating liver to be lower than in resting liver. It is not unreasonable to expect interspecies differences in the enzyme patterns of regenerating liver. A recent report, however, describes the elevation of liver xanthine oxidase levels during regeneration following carbon tetrachloride administration to rats (27).

It has been shown that dietary deficiencies produce sharp declines in liver xanthine oxidase levels (16–18, 28). It is quite significant, therefore, that although the hepatectomized animals probably had a low food intake after the surgical trauma and showed significant body weight loss and depletion of total hepatic nitrogen, the xanthine oxidase activity in regenerating liver nevertheless was elevated.

RNA and DNA are synthesized at greater rates in regenerating than in resting livers (4, 9, 17, 19, 21, 26). It is apparent that liver xanthine oxidase activity and nucleic acid synthesis follow parallel courses during liver regeneration, and consequently that a causal relationship may exist between these phenomena. Support for such a contention is found in the work of Dinning (6, 7) who reports that a vitamin E deficiency in rabbits promotes a considerable increase in both liver xanthine oxidase level and nucleic acid turnover. It is possible to postulate a mechanism whereby the augmented nucleic acid turnover in regenerating liver may be responsible for elevated xanthine oxidase levels *via* adaptive enzyme formation induced by the purines provided by the increased nucleic acid turnover. Compatible with this proposal is the report of Begg and Burton (3) who demonstrate a xanthine oxidase elevation in protein-depleted animals in response to dietary administration of nucleic acids. In this way, high levels of xanthine oxidase in regenerating liver may partake in carrying the increased catabolic load due to the elevated nucleic acid turnover in this tissue. Conversely, it may be suggested that xanthine oxidase, although traditionally considered to be a catabolic enzyme, may play a role in implementing nucleic acid synthesis.

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