The Mechanism of Dietary Alterations in Rat Hepatic Xanthine Oxidase Levels*

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SUMMARY

Dietary protein depletion results in a decrease in rat hepatic xanthine oxidase activity to about 10% of control levels. Refeeding of a high protein diet leads, after a 6-hour lag phase, to a 5-fold increase in enzyme activity by 12 hours. The mechanism of this increase was studied utilizing actinomycin D, 5-fluorouracil, and puromycin. These agents all blocked the increase in enzyme activity, indicating that both ribonucleic acid and protein syntheses were involved. Following administration of 14C-leucine, specific radioactivities of xanthine oxidase were identical in the livers of protein-depleted and control animals, despite a 10-fold difference in enzyme activity levels. These results indicated that the fractional turnover rates were the same in the two groups of rats and suggested that the rate of xanthine oxidase synthesis was reduced 10-fold in the depleted rats prior to protein refeeding. Greatly increased isotopic labeling of highly purified enzyme with 14C-leucine during the phase of restoration of enzyme levels indicated that the increase in activity was the result of accelerated apoenzyme synthesis de novo.

In 1948 Miller (1) reported a marked decrease in activity of xanthine oxidase in the livers of rats that were fasted or fed low protein diets. In typical experiments (2), he noted a decrease of xanthine oxidase activity to 15% of control levels after 9 days on a 6% protein diet. After 72 hours of refeeding a high protein diet, enzyme activity was restored to 75% of control levels. These findings have been confirmed by many workers (3) and, in addition, restrictions of dietary protein or starvation have been shown to cause marked changes in levels of activity of a number of other enzymes, some of which are increased rather than decreased in activity (4).

The mechanisms controlling these variations in enzyme activity in mammalian species have been studied in detail in only a limited number of instances. Among the mechanisms which could operate to establish new levels of enzyme activity in response to dietary changes are alterations in rates of enzyme protein synthesis or degradation, production or destruction of enzyme inhibitors or activators, or regulation of supply of co-factors. There is need for additional careful study of model systems capable of throwing light on the problem of enzyme homeostasis in complex organisms, which may well differ in important ways from the model systems so well exploited in bacterial systems.

The present study was undertaken to define some of the factors regulating xanthine oxidase levels in rat liver. The increase in xanthine oxidase activity on refeeding of protein was found to require new ribonucleic acid and new protein synthesis, and specifically to involve the synthesis of new xanthine oxidase protein. Thus, one important factor in the regulation of xanthine oxidase levels by protein intake is the regulation of the rate of enzyme synthesis.

EXPERIMENTAL PROCEDURE

Materials

5-Fluorouracil was obtained from Hoffmann LaRoche; actinomycin D (Lyo-Meractinomycin) from Merck Sharp and Dohme; puromycin, inosine, hypoxanthine, xanthine, and 8% "Low Protein" diet from Nutritional Biochemicals; orotic acid-2,14C (specific activity, 26 mC per mmole) from Calbiochem; and L-leucine-14C (specific activity, 220 mC per mmole) from Schwarz Bioresearch.

Methods

Dietary Regimen

Female Osborne Mendel rats, 10 to 11 weeks old, weighing 170 to 200 g, were used in all experiments. These animals had been maintained exclusively on standard Purina rat chow (23% protein) from weaning. Where possible, litter mates were used for each experiment. The rats were housed five to a cage, and for 14 days each received approximately 14 g of 8% low protein diet. The sole protein source in this diet was casein. The diet contains salt mixture USP XIV and Vitamin Diet Fortification Mixture (Nutritional Biochemicals). On this regimen the animals gained 5 to 10% of their original weight over 14 days compared with a 10 to 15% gain for control animals maintained on standard Purina rat chow. Refeeding was carried out with Purina rat

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chow after an overnight fast to ensure adequate food intake. The rats were observed to eat the proffered food promptly. Animals were killed in three groups, i.e., prior to refeeding and at 6 and 12 hours after refeeding. It was found that the major rise in enzyme activity occurred in this first 12-hour period, and that the longer 72-hour period used in other studies was unnecessary.

In an attempt to assess the effect of inosine feeding on enzyme levels, inosine, in an approximate daily dosage of 50 mg per rat, was admixed with the low protein diet over the 14-day period.

**Assay Techniques**

**Xanthine Oxidase—**Animals were killed by stunning and decapitation, following which, the livers were removed and placed in ice-cold 0.1 M Tris at pH 8.1. The livers were blotted of excess buffer, dissected free of any attached muscle, peritoneum, or fibrous tissue, and weighed. Homogenization of whole liver was carried out in 5 ml of ice-cold 0.1 M Tris at pH 8.1 per g of liver for 45 sec in a Potter-Elvehjem homogenizer fitted with a motor-driven Teflon pestle.

Twenty milliliters of homogenate were sonically treated for 2 min (Branson Sonifier, 20 kc, 3.4 to 3.8 amperes) in a 5-cm diameter centrifuge tube embedded in ice. The conditions and time period of sonic treatment were critical for maximum specific enzyme activity. The sonically treated homogenate was centrifuged at 600 × g for 20 min at −4°. The supernatant solution was spun at 105,000 × g for 60 min at 4°, the layer of lipid was carefully removed, and the remaining supernatant solution was dialyzed against three changes of 100 volumes of 0.1 M Tris at pH 8.1 for 3 hours at 4°.

The dialyzed solution was assayed for xanthine oxidase activity at 25° by a modification of Morell's (3) adaptation of the technique of Kalckar. The technique employs measurement of the rate of change of optical density at 292 mμ due to the appearance of uric acid after the addition of xanthine substrate. Twenty milliliters of dialyzed solution was added to 0.6 ml of 0.3 mM xanthine and 2.2 ml of 0.136 mM Tris, pH 8.1. The final concentration of xanthine was 60 μM and of Tris 0.1 M. The pH of the reaction mixture was 8.3. The rate of change of optical density was recorded on a Gilford multiple sample absorbance indicator connected to a Beckman DU ultraviolet spectrophotometer, and the enzyme activity was expressed as the change in optical density per mg of protein.

**RNA—**RNA was extracted in the form of bases employing the Fleck and Munro (6) modification of the Schmidt-Thannhauser procedure. This was again slightly modified by placing the liver sample directly into ice-cold 10% perchloric acid. The bases were quantitatively measured by measuring ultraviolet absorption at 260 mμ.

Orotic acid-2-14C was used to label RNA. Animals were given a 2-hour pulse of 2.5 μC (specific activity, 26 μC per mmole) prior to death. A 2-hour pulse gave adequate, but by no means maximal, labeling of RNA. Radioactivity was measured by counting aliquots of the RNA hydrolysate in one part of Triton X 100 to four parts of toluene-2,5-diphenyloxazole-1, 4-bis[2-5-phenyl-oxazolyl]benzene (POPOP-PPOPOP) in a Packard Tri-Carb liquid scintillation spectrometer.

**Protein—**This was determined by the method of Lowry et al. (7) with crystalline bovine serum albumin as a standard.

**Purification of L-Leucine-14C-labeled Xanthine Oxidase (Table I)**

—This procedure is a modification of those of Kielley (8) and Rajagopalan.1 Leucine constitutes 8.5% of the amino acid residues of xanthine oxidase (9). A 0-hour pulse of 8 μC (0.04 μmole) was given intraperitoneally to label the enzyme. The livers of 10 animals were pooled for each purification procedure. These were homogenized in ice-cold 0.05 M phosphate buffer, pH 7.8, containing 0.1 mM EDTA, 5 ml per g of liver, in a Potter-Elvehjem homogenizer fitted with a motor-driven Teflon pestle.

The homogenized solution was sonically treated in a Teflon beaker embedded in ice utilizing a Branson Sonifier (20 kc, 3.4 to 3.8 amperes). The sonic treatment was performed in 2-min bursts to avoid overheating. After centrifugation at 600 × g for 20 min, the volume of the supernatant fluid was reduced to 340 ml, and centrifugation at 105,000 × g for 1 hour reduced this to 250 ml.

### Table I

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Volume</th>
<th>Protein concentration</th>
<th>Enzyme specific activity</th>
<th>260/450 O.D. ratio</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>105,000 × g centrifugation</td>
<td>250</td>
<td>21.8</td>
<td>5.18</td>
<td>9.1</td>
<td>100</td>
</tr>
<tr>
<td>Heating to 60° for 2 min</td>
<td>202</td>
<td>4.0</td>
<td>25.0</td>
<td>41.0</td>
<td></td>
</tr>
<tr>
<td>Ammonium sulfate fraction</td>
<td>50</td>
<td>0.8</td>
<td>50.0</td>
<td>34.3</td>
<td></td>
</tr>
<tr>
<td>Acetone fraction and dialysis</td>
<td>50</td>
<td>4.62</td>
<td>67.5</td>
<td>12.2</td>
<td>31.5</td>
</tr>
<tr>
<td>Hypatite column and ammonium sulfate fraction</td>
<td>5</td>
<td>0.45</td>
<td>2000</td>
<td>5.18</td>
<td>0.1</td>
</tr>
</tbody>
</table>

1 K. V. Rajagopalan, personal communication.
standard 0.05 M phosphate buffer at pH 7.8 containing 0.1 mM EDTA.

Acetone extraction was performed by the slow addition, with constant stirring, of ice-cold acetone to a concentration of 50% (v/v). Stirring was continued for 15 min, and the solution was centrifuged at 10,000 \( \times g \) for 30 min. The precipitate was dissolved in 50 ml of the standard phosphate buffer. Any unsolved material was removed by centrifugation at 2,000 \( \times g \) for 30 min. The resulting pale yellow solution was dialyzed against two changes of 100 volumes of equilibration buffer for 3 hours.

A hydroxylapatite (Hypapite C, Clarkson Chemical) column (15.0 \( \times 1.0 \) cm) was utilized in the most crucial step of the purification procedure. The column was prepared by pouring a suspension of hydroxylapatite in 0.05 M phosphate buffer at pH 7.8 (no EDTA) into a glass chromatographic tube (1-cm internal diameter, fitted with a sintered glass disc) previously half-filled with buffer. The column was packed carefully under 5 p.s.i. pressure. The dialyzed solution (50 ml) was added and allowed to adsorb onto the column. The column was then washed successively with 50 ml each of 0.05 M, 0.075 M, 0.1 M, 0.15 M, and 0.2 M phosphate buffer at pH 7.8 under 5 p.s.i. pressure. The enzyme was eluted with the 0.2 M buffer. By carefully collecting small (2 ml) fractions, one can obtain samples of highly purified enzyme. The most highly purified fractions were pooled and ice-cold neutralized ammonium sulfate was added with constant stirring to 60% of saturation in order to precipitate the enzyme. Centrifugation at 105,000 \( \times g \) for 30 min was required to collect the enzyme which was then dissolved in 5 ml of 0.05 M buffer. The enzyme solution was then dialyzed for 2 hours against two changes of 100 volumes of the buffer.

In terms of enzyme specific activity, the over-all purification from the stage of the 105,000 \( \times g \) supernant fluid to the final preparation was some 200-fold in the case of control animals and 2000-fold in the case of protein-depleted animals. The final specific activities, and 280 to 450 O.D. ratios were all in the same range for control, protein-refed, and protein-depleted animals. Quantitatively, however, the total activity recovered in the form of the purified enzyme was proportional to that present in the original homogenate in each group of test animals.

The technique had an over-all yield of approximately 16%. The best 280 to 450 \( \mu \)O.D. ratio was 5.18, a figure closely approximating that obtained by Brumby in preparing highly purified xanthine oxidase from pig liver for ultracentrifugal studies.

**RESULTS**

**Effect of Diet on Xanthine Oxidase Activity**—Table II presents mean values of a series of experiments, all of which gave essentially the same results. The specific activity of xanthine oxidase fell rapidly over the first 10 days on an 8% protein diet and reached a steady state level of about 10% of control values at 14 days. Further feeding of a restricted protein diet for periods of up to 4 weeks did not result in any further decrease in activity. The total hepatic activity was reduced to a greater extent, as the liver weight per 100 g of rat body weight was decreased some 16% by dietary protein restriction.

Refeeding of a 23% protein diet resulted in a rise of activity to levels about 55% of control levels in 12 hours. There was an initial 6-hour lag period followed by a rapid rise between 6 and 12 hours. Peraino, Blake, and Pitot (10) showed a similar lag phase in the induction of serine dehydrase and ornithine transaminase with the use of a similar dietary technique in rats. The rise over the 12-hour period represented a 5-fold increase in xanthine oxidase activity.

When varying mixtures of homogenate from control, diet-treated, and refeed animals were assayed, the total activity was equal to the sum of the individual activities. The result would seem to exclude the presence of an enzyme inhibitor or the absence of an enzyme activator in the protein-depleted state.

**Changes in Hepatic Protein (Table II)**—During 14 days on protein restriction, total hepatic protein decreased 16% almost in parallel with the decline in liver weight per 100 g of body weight. The percentage of liver weight that was protein was relatively unchanged. Upon refeeding the 23% protein diet, little change occurred in hepatic protein content during the first 12 hours when the rapid rise in enzyme activity occurred, but thereafter it rose progressively and reached normal values in 72 hours, paralleling the increase in liver weight which occurred.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Time on diet</th>
<th>Change in liver weight per 100 g body weight compared with controls</th>
<th>Protein concentration</th>
<th>Liver enzyme activity</th>
<th>Liver enzyme activity</th>
<th>Liver enzyme concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>8% protein</td>
<td>0</td>
<td>0.0%</td>
<td>125</td>
<td>1443</td>
<td>5050</td>
<td>11.5</td>
</tr>
<tr>
<td>1 day</td>
<td>-1</td>
<td>125</td>
<td>1417</td>
<td>4910</td>
<td>11.3</td>
<td></td>
</tr>
<tr>
<td>5 days</td>
<td>-12</td>
<td>123</td>
<td>713</td>
<td>2296</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>7 days</td>
<td>-11</td>
<td>122</td>
<td>476</td>
<td>1483</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>10 days</td>
<td>-14</td>
<td>121</td>
<td>145</td>
<td>436</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>14 days</td>
<td>-16</td>
<td>120</td>
<td>144</td>
<td>423</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>22 days</td>
<td>-18</td>
<td>118</td>
<td>136</td>
<td>390</td>
<td>1.15</td>
<td></td>
</tr>
<tr>
<td>28 days</td>
<td>-22</td>
<td>117</td>
<td>143</td>
<td>390</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>28% protein</td>
<td>14 days on 8%</td>
<td>121</td>
<td>151</td>
<td>449</td>
<td>1.25</td>
<td></td>
</tr>
<tr>
<td>24 hr</td>
<td>-14</td>
<td>123</td>
<td>701</td>
<td>2110</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>72 hr</td>
<td>-12</td>
<td>122</td>
<td>856</td>
<td>2727</td>
<td>6.9</td>
<td></td>
</tr>
</tbody>
</table>

* Mean value is 3.54 g/100 g of body weight.
RNA, was evaluated. 5-Fluorouracil was given intraperitoneally in a dose of 50 mg hourly, commencing at the time of refeeding, into the RNA molecule leads to formation of biologically inactive RNA which does not inhibit RNA synthesis, but by being incorporated with an inhibition of the rise in enzyme activity. If, however, the administration of actinomycin was delayed until 6 hours after refeeding, and its administration at commencement of refeeding was also associated (Table IV) with an inhibition of the rise in enzyme activity. It was found to block the anticipated rise in activity of xanthine oxidase completely. This result indicated that new protein synthesis was also a requirement of the restoration of xanthine oxidase activity associated with refeeding of protein.

Synthesis of New Xanthine Oxidase Protein—The results reported above indicate that both new RNA and new protein synthesis in order to maintain constancy of enzyme levels and activity were required for the rise of xanthine oxidase activity but do not prove that new xanthine oxidase has been synthesized. Other possibilities include a dependence upon a newly synthesized protein for synthesis of coenzyme, or for the insertion of cofactor into inactive apoenzyme, or perhaps for the assembly of inactive enzyme subunits.

For this reason existing methods were modified so as to isolate highly purified xanthine oxidase from rat liver, and the rate of incorporation of L-leucine-2-14C into xanthine oxidase was studied in rats reeled protein diets and in those refed with the 23% protein regimen. Eight microcuries (approximately 0.04 μmole) were given intraperitoneally 6 hours after refeeding at the time when the rise in xanthine oxidase was expected to begin, and the animals were killed 6 hours later.

Table V shows that the incorporation of L-leucine into xanthine oxidase was 4 times greater in refed animals than in control animals or in protein-depleted animals that had not been refeed. The specific radioactivities of enzyme in the two groups of animals studied under steady state conditions were almost identical.

The amino acid pools in livers of protein-depleted rats are not appreciably different, in milligrams per g of tissue, from those of fed animals (12). Therefore, the dilution of the labeled leucine was probably about equal in the control and depleted animals. The finding of identical specific radioactivity values of xanthine oxidase in the two groups implies identical fractional turnover rates in the two sets of rates. Since a new steady state has been reached with respect to enzyme levels at the time of study of the protein-depleted rats, it appears unlikely that an accelerated rate of destruction of xanthine oxidase was present, for such a circumstance would require a corresponding increase in rate of synthesis in order to maintain constancy of enzyme levels and would have led to an increase in specific radioactivity and fractional turnover rate. Since the control group had levels of xanthine oxidase 10-fold greater than the protein-depleted group, these data imply that the rate of synthesis of xanthine oxidase was reduced to one-tenth the control level in the latter animals.

One would anticipate that protein refeeding of depleted animals might temporarily increase the hepatic amino acid pools,
Table V

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>280/450 mp O.D. ratio</th>
<th>Enzyme activity</th>
<th>Enzyme specific radioactivity</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>5.18</td>
<td>2000</td>
<td>170</td>
</tr>
<tr>
<td>B</td>
<td>5.24</td>
<td>3260</td>
<td>195</td>
</tr>
<tr>
<td>Diet alone (8% protein)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>5.25</td>
<td>2000</td>
<td>158</td>
</tr>
<tr>
<td>B</td>
<td>5.25</td>
<td>3180</td>
<td>200</td>
</tr>
<tr>
<td>Refed 12 hours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>5.26</td>
<td>2100</td>
<td>775</td>
</tr>
<tr>
<td>B</td>
<td>5.28</td>
<td>3245</td>
<td>900</td>
</tr>
</tbody>
</table>

Discussion

Enzyme purification and isotope incorporation data on three experimental groups and results of two different experiments, A and B, are shown. In each, control animals were maintained on a 25% protein diet. The treated group was fed an 8% protein diet for 14 days. The refed group, which had been maintained for 14 days on an 8% protein diet, was killed 12 hours after receiving a 25% protein diet; 280 to 450 mp O.D. ratios indicate a close correlation between these groups with regard to the degree of enzyme purity. Enzyme activity is calculated as the change in optical density per min per mg of enzyme protein. Isotope incorporation is measured as counts per min per mg of enzyme protein. There was a 4-fold increase in specific radioactivity of the enzyme during refeeding compared with controls and diet treated animals.

unless the rates of protein synthesis and leucine metabolism were delicately synchronized with the rate of leucine entry into liver cells. Under conditions of enhanced enzyme synthesis the turnover of the leucine pool may have been accelerated, but it is to be noted (Table II) that the major increase in xanthine oxidase activity occurs prior to a significant change in total protein levels in liver. Although direct measurements of the specific activity of the precursor leucine pool were not made, these factors suggest that the specific radioactivity of xanthine oxidase during protein refeeding, approximately 4-fold that found in the depleted or control rats, is probably a minimal one. Since the enzyme activity levels reached about 50% of control values at the end of the leucine pulse, it seems likely that the absolute rate of xanthine oxidase synthesis was at least twice as great in the refed rats during the restoration phase as in the nondepleted control rats, and perhaps 20 times as great as in the depleted animals. These interpretations do not exclude the possibility that alterations of rates of degradation or excretion of enzyme may have played a role in the decrease of activity of xanthine oxidase that follows a shift to a protein-restricted diet.

Effect of Substrates and Substrate Precursor—The feeding of inosine in conjunction with the 8% protein diet failed to prevent the drop in enzyme activity. Furthermore, the rise in enzyme activity produced by refeeding could not be produced in protein-restricted animals by the intraperitoneal injection of xanthine, hypoxanthine, or inosine in doses of 50 mg given at varying peri-ods up to 24 hours prior to death. The free bases, however, were relatively insoluble and poorly absorbed. Hence, the rise, if any, in intracellular concentration may not be sufficient to produce an effect.

Discussion

In bacterial systems significant changes in enzyme activities generally arise from changes in gene action brought about by the addition or removal of inducers, resulting in changes in rate of synthesis of messenger RNA and of enzyme protein. Since the half-life of cell division is short compared with the half-life of the enzyme, reductions of enzyme activity following the withdrawal of inducer from growing cultures reflect dilution to a very much larger extent than enzyme turnover.

In mammalian systems, enzyme levels may often be affected by dietary measures or by drug administrations, but often the magnitudes of change are small compared with those observed in bacteria. In a number of instances what superficially appeared to be enzyme induction has turned out to be enzyme stabilization by substrate. Also, the ratio of cell life span to enzyme turnover time is very much greater than in bacteria. As a consequence, enzyme degradation has been regarded as a more important regulatory factor in mammalian than in bacterial systems, and the role of gene regulation and protein synthesis in the control of enzyme levels has been considered by some authors (13, 14) to be a minor one.

The present study indicates that under certain circumstances major changes in enzyme activity can be attributed to regulatory phenomena which appear to operate at the level of the gene. The return of low levels of hepatic xanthine oxidase toward normal shortly after refeeding of protein-depleted rats with a high protein diet has all the features of genetic induction. There is a dependence upon new RNA and new protein synthesis, and if new RNA is allowed to form for a time following refeeding (hepatic RNA increases within 3 hours of refeeding (15)), the subsequent administration of actinomycin D does not block the increase of xanthine oxidase levels. The increase of xanthine oxidase activity was shown to involve new synthesis of xanthine oxidase apoenzyme.

It is true that xanthine oxidase is an enzyme of great complexity, the catalytic activity of which is dependent upon functional participation of protein-bound FAD, molybdenum, and nonheme iron. Deficiency of iron or molybdenum results in decreased levels of xanthine oxidase activity in experimental animals (16). Although no experiments on the mechanism of decline of xanthine oxidase activities in protein depletion were conducted in the present study, dietary deficiency of iron or molybdenum is not the likely explanation, as both the 8% and the 25% protein diets contained more than adequate amounts of these elements. The possible formation of a pool of inactive enzyme, which did not behave as active xanthine oxidase during purification, would not invalidate the conclusion of accelerated enzyme synthesis on protein refeeding. Such a hypothetical pool, if present, would possibly dilute the specific radioactivity of new enzyme, in which case the 4-fold difference recorded would lead to an underestimation of the actual increment of enzyme synthesis on refeeding.

A number of other examples exist which also suggest that variations in rates of enzyme synthesis underlie the differences in steady state levels of enzymes observed with dietary manipulations. Schimke (4) reported a fall in activity of five enzymes of the urea synthetic pathway in rat liver with dietary protein restriction which contrasted with a 3-fold rise in activity of these enzymes when animals were fasted for 7 days. His evidence was suggestive that alterations in rates of enzyme protein synthesis do novo accounted for the variations of activity. Peraino et al. (10), using techniques of protein depletion and repletion similar to those employed in the present study, demonstrated what appears to be true induction of threonine dehydrase and ornithine dehydrase.
thine transaminase in rat liver. By means of isotopic and immunological techniques, Schimke, Sweeney, and Berlin (17) showed that the increased activity of rat hepatic tryptophan pyrrolase following cortisone administration was due to enzyme protein synthesis, a conclusion also suggested by the work of Garen et al. (11), in which, however, stabilization of enzyme by tryptophan (17) was not rigorously excluded as a factor leading to an appearance of induction. Finally, increase in activity levels of δ-amino levulinate synthetase following treatment of chick embryo liver cultures with allylisopropylacetamide, and the inhibition of this rise by actinomycin D, mitomycin, and puromycin, also have all the characteristics of induction, although isotopic, immunological, or physical isolation studies have not yet proved the point in a definitive manner (18).

The evidence is increasing that control mechanisms operating at the level of the gene are of major importance in the regulation of levels of enzyme activity in mammalian systems, although such mechanisms are necessarily of much slower responsiveness than those which regulate the catalytic activity of existing enzyme molecules. The sensitivity of responsiveness is, however, appropriate to the stimulus; in the case presented the reduction of activity of xanthine oxidase to minimal levels required 10 days of protein depletion, whereas restoration was half-completed within 12 hours of protein refeeding.

The biological significance of the variation in xanthine oxidase with protein intake is not yet clear. We have confirmed the observation of Bass et al. (19) that there is no reduction in purine catabolism, as reflected in allantoin excretion, as a consequence of the fall in xanthine oxidase activity. Nor is there any information yet available on the nature of the chemical signal withdrawn or introduced by low and high protein diets that controls the genetic apparatus governing xanthine oxidase synthesis. Presumably, it is the synthesis of messenger RNA for xanthine oxidase that is controlled, but other mechanisms involving syn-

2 P. B. Rowe and J. B. Wyngaarden, unpublished observations.

REFERENCES

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