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An Electron Microscopic and Enzymic Study of Rat Liver Peroxisomal Nucleoid Core and Its Association With Urate Oxidase

Gene F. Lata, Frank Mamrak, Phillip Bloch, and Barbara Baker

Department of Biochemistry, University of Iowa, Iowa City, Iowa 52242

The appearance of the characteristic crystalloid core of rat liver peroxisomes is emulated by the electron microscopic (EM) appearance of highly purified urate oxidase prepared from the same tissue. The purity of the enzyme preparation was established by gel electrophoresis under various conditions and the specific enzyme activity was at least as high as any previously reported. The amino acid composition of urate oxidase was determined. As additional evidence for close association of the peroxisomal core with urate oxidase, it was demonstrated that the biphasic changes in rat liver urate oxidase activity in response to prolonged starvation were paralleled by changes in the EM appearance of peroxisomes. Under comparable conditions catalase, another peroxisomal enzyme, did not show the same changes in activity as did urate oxidase. Evidence for the possible identity of urate oxidase with the peroxisomal crystalloid of rat liver has been presented, all materials having been obtained from, and experiments performed with, the rat.

Key words: peroxisome, microbody, nucleoid core, urate oxidase, starvation effects, rat liver enzymes, catalase, cell organelle

The characteristic presence of a dense crystalloid core, or nucleoid, in rat liver peroxisomes is a long-standing observation (1). Association of urate oxidase activity (urate: O_2 oxidoreductase, EC 1.7.3.3) with this nucleoid has been previously indicated (2–5). The evidence for this association has been in part histochemical. More directly, it has been demonstrated that only urate oxidase activity remains after purification of the peroxisomal core (5) subcellular fraction. Electron microscopy of rat liver peroxisomal nucleoid preparations by Tsukada et al (6) revealed a similarity in their appearance compared to that of the crystalloid core of in situ organelles, but the nucleoids were not isolated by these authors using criteria of protein/enzymic purification. In an earlier report of nucleoid-like electron microscopic (EM) appearance of a urate oxidase preparation (7), a commercial preparation was used and with no evaluation of purity.

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Frank Mamrak is now at E.R. Squibb Research Institute, New Brunswick, New Jersey, Phillip Bloch is now at the Department of Microbiology, University of Michigan, Ann Arbor.

If there is a necessary association of urate oxidase activity with the nucleoid core, and the possible identity of the two is to be tested, urate oxidase must be purified to protein homogeneity and its EM appearance compared with the in situ peroxisomal core of the same tissue. In addition, under conditions where in total rat liver urate oxidase activity shows marked variation, there should be demonstrable a parallel change in the appearance of the in situ peroxisome. In this report we described an EM examination of urate oxidase, purified by a modification of our previously reported procedure (8); due to the insolubility of this enzyme it was necessary to develop new solvent systems to test for protein and enzymic homogeneity. The amino acid composition of enzymes prepared from male and female animals was compared and found similar, a finding consistent with indications of high purity. We have also demonstrated parallel changes in the in situ appearance of the peroxisomal core and starvation-induced changes in total rat liver urate oxidase activity. For comparison the effect of starvation on the activity of catalase, another peroxisomal enzyme, was also determined.

A preliminary report of a portion of this work has been presented (9).

MATERIALS AND METHODS

Uric acid was recrystallized from water; 30% hydrogen peroxide was diluted directly before use. Actinomycin D (Merck, Sharp and Dohme) was obtained dissolved in aqueous 1.8% mannitol. Urea was recrystallized from 95% ethanol at -20° C, washed with dry ether, and dried in air. The urea solutions were, when necessary, stored at 5° but for no longer than one week before use. The t-butanol and t-pentanol was redistilled and stored at room temperature. All water was redistilled, and all other materials were of reagent grade, used without further purification.

Assay of Tissue Enzyme Activities

Sprague-Dawley male or female rats of the Simonsen or Sasco strain (120–200 gm) were used, caged individually with free access to water. At midday (noon) animals were decapitated with a guillotine, and the liver was exposed and perfused in situ with cold 0.9% sodium chloride. In experiments involving electron microscopic examination, a portion of each lobe was sampled for EM preparation and the remainder of the liver homogenized; total liver weight was recorded. The liver tissue was homogenized in a Potter-Elvehjem homogenizer using 0.22 M sucrose, 0.001 M EDTA, 0.01 M potassium phosphate, pH 7.4 solution to give a 10% homogenate. This was filtered through a stainless steel wire screen and the filtrate was centrifuged at 2,000g for 10 min at 0°. The pellet was discarded. This first supernatant fluid was recentrifuged at 2,000g for 10 min and the second pellet resuspended in 0.2 M borate (pH = 8.3) buffer.

Where indicated an alternative sampling procedure was followed in which the perfused liver was homogenized in Krebs-Ringer phosphate buffer, pH 7.1, to give a 10% homogenate.

The suspended pellets or supernatant fluids were assayed spectrophotometrically for urate oxidase, by ΔA_{292} of a urate solution (10). The Krebs-Ringer phosphate homogenates were diluted tenfold and assayed colorimetrically as described by Leeling and Lata (11).

For catalase assay 5 ml of a 1% homogenate was treated with 0.05 ml 95% ethanol (to decompose inactive H_2O_2-catalase complexes) and with 0.55 ml 10% Triton X-100 (to solubilize the enzyme). This preparation (0.5 ml) was mixed with 50 ml of 6×10^{-3}

MH_2O_2 at 4°C and the substrate disappearance was determined colorimetrically after three minutes according to the method of Cohen et al (12). At 4°C the disappearance of H_2O_2 was linear with time up to 5 minutes under the conditions used and at all enzyme levels encountered.

Protein concentrations were determined by the Lowry (13) or the biuret (14) methods. Enzyme results were calculated as μmoles substrate utilized per min•mg protein. Spectrophotometry and colorimetry were performed with a Beckman DB-G spectrophotometer.

Electron Microscopy

Pieces of rat liver and the precipitated urate oxidase were fixed in 2.5% glutaraldehyde in 0.1 M Sörenson's phosphate buffer at pH 7.4, and postfixed in 1% osmium tetroxide in Sörenson's phosphate buffer at pH 7.4 for one hour, then dehydrated in acetone and embedded in Vestopal W (15) as usual. Thin sections were cut on a Sorval MT2-B Ultra-Microtome equipped with a diamond knife. Gray to light gold sections were mounted on 300- or 400-mesh copper grids. Staining was done with uranyl acetate (16) followed by lead citrate (17). The sections were then examined in a Hitachi HU-125E electron microscope.

Starvation Experiments

Rats were kept in individual cages with free access to water. Six groups of aminals were used, including the ad lib fed control; starvation periods were for 1, 2, 3, 4, and 5 days. Actinomycin D was injected IP at the indicated doses and various times during starvation.

Enzyme Purification

Urate oxidase from male and female rats was purified by a modification of the previously described method (8). This procedure involved separation of the "mitochondrial-peroxisomal" fractions of rat liver followed by selection extractions by sucrose, borate, phosphate, deoxycholate-borate, and finally sodium carbonate-t-butanol buffers. The final extraction of urate oxidase was into 0.15% Na_2CO_3 –6% (v/v) t-butanol solution, pH = 11. The pH of the enzyme solution was carefully adjusted to 9.5 with HCl at T = 5°C; a white precipitate formed in about five minutes. After standing for an additional 30 minutes at 5°, the mixture was centrifuged at 37,000g for 20 minutes, the supernatant fluid discarded, and the pelleted enzyme reserved for later use. Standard disc gel electrophoresis of the redissolved enzyme indicated homogeneity as previously demonstrated (8).

Sulfhydryl Analysis

The method described by Elman (18) was modified by the use of 10^{-4} M EDTA in the reagent mixture.

Disc Gel Electrophoresis

Acrylamide gels (5.3%, 7.5%, and 10%) were prepared according to Clarke (19) with the following modifications: 8 μl N_4-tetramethylethylenediamine was used for the non-urea gels and 4 μl of this reagent for the urea-containing gels; 17 mg ammonium persulfate was added after the mixture was brought to a final volume of 24.0 ml with one of the following buffers: A) 0.1 M tris-acetate, 5% (v/v) t-pentanol, pH = 9.5–10.6; B) 0.1 M tris, 15% (v/v) t-butanol, pH = 10.6; C) 0.1 M tris-acetate, 12.8% (v/v) acetonitrile, 8.6% (v/v)

t-pentanol, pH = 8.50–9.50; D) 0.039 M glycine, 0.0050 M tris-HCl, 8.0 M urea, pH = 8.9; E) 0.005 M glycine, 8.0 M urea, pH = 9.7, adjusted with NaOH. The remainder of the procedure was carried out as previously described, including protein and activity staining.

Bidirectional Electrophoresis

The sample chamber was a one-inch piece of Nalgon tubing (3/8-inch inside diameter) with a 3-mm hole in the side (stoppered with a Nalgon plus) for introduction of the sample. Tubes containing 5% gels were inserted into both ends of the sample chamber and the latter was filled (0.1–0.3 ml) with a syringe. In these systems the same buffer was used for preparation of the gel, sample solution, and electrolyte in the electrode compartments. The two gel tubes, connected by the sample chamber, were mounted in the electrophoresis unit so that one tube was immersed in the cathodic and the other in the anodic electrolyte chamber. This procedure permitted the detection of proteins migrating either as anions or cations.

Molecular Weight Estimation by SDS Acrylamide Gel Electrophoresis

The procedure was essentially that of Weber and Osborn (20). The marker proteins were obtained from Mann Corp. The protein bands were visualized with Coomassie blue (2.5% dye in 50% methanol, 9% acetic acid). A departure from the original procedures was the use of a 1:1 (v/v) mixture of the phosphate buffer and of 0.15% Na_2CO_3–6% (v/v) t-butanol to dissolve the proteins.

Amino Acid Analysis

Samples of precipitated protein (3–4 mg), after deoxygenation and nitrogen flushing, were hydrolyzed in 12 N HCl for 12, 22, 70, and 144 hours at 100°C. The hydrolysates were analyzed in a Beckman 120 C amino acid analyzer using standard procedures.

Fluorescence Measurements

Fluorescence measurements were made with a Hitachi Perkin-Elmer Fluorescence Spectrophotometer Model MPF-2A, with sample cell temperature controlled by a Lauda K-2/R thermostated circulating water bath.

RESULTS

Solubility of Urate Oxidase Preparations

The purified enzyme preparation was soluble in 0.1 M tris at pH 10.6 and 25°C if t-butanol (15% v/v), or t-pentanol (5% v/v) were added. If the pH was decreased to 9.5 or below the enzyme precipitated. The presence of acetonitrile (12.8% v/v) and t-pentanol (8.6% v/v) in 0.1 M tris permitted the pH to be lowered to 8.4 without precipitation.

Lowering of the temperature to 5°C resulted in precipitation of the enzyme from all solutions at all pH's. The precipitate could not be redissolved by increasing the temperature to 25°C unless t-pentanol (2.3–5% v/v) was present in the solvent. Reversible precipitation from such t-pentanol-containing solvents could be effected with full retention of enzyme activity. These precipitates were used for the electron microscope studies reported below.

If dithiothreitol at concentrations up to 30 mM were added to a solution of urate oxidase dissolved in 0.1 tris, 5% (v/v) t-pentanol (pH 10.6), a precipitate formed immedi-

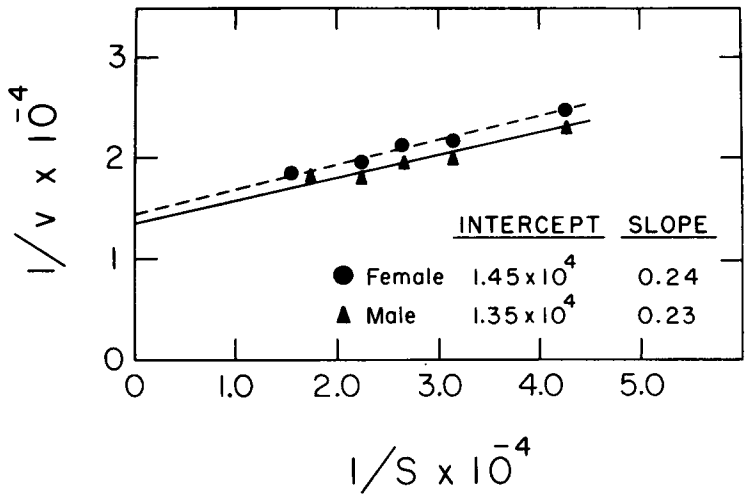


Fig. 1. Reciprocal plot of kinetic data for urate oxidase prepared from female • and male ▲ rat livers. Activity units are described in the text. The abscissa is expressed as reciprocal moles of uric acid.

ately; β-mercaptoethanol has a similar effect at concentrations up to 45 mM. If 8 M urea were also present, however, no precipitation occurred, and as indicated below, these were one set of electrophoretic conditions used.

Comparison of Urate Oxidase Activities From Male and Female Rats

Purified enzymes from male and from female rats prepared without precipitation and evidencing the preciously reported sex difference in activity (8) were precipitated by pH adjustment and the precipitates redissolved in 0.15% Na_2CO_3, 6% (v/v) t-butanol (pH = 11.1). These urate oxidase solutions no longer showed sex differences in specific activity. If the pH's of the supernatant fluids from these precipitations were readjusted to 9.5 or greater and the precipitated enzymes redissolved, the activities were again the same. In a typical experiment the specific activity of both the male-derived and female-derived enzymes, after precipitation and resolution, was 13 units per mg protein; initial activities were 9.5 (male) and 12.0 (female). The kinetic constants for the two preparations obtained from a Lineweaver-Burk plot (Fig. 1) were also identical. The V_{max} values were 14.1 μmoles·min^{-1}·mg^{-1} for the enzyme obtained from male rat livers and 15.0 μmoles·min^{-1}·mg^{-1} for the enzyme from female livers. The Km values were 3.2×10^{-6} M for the female in the substrate concentration range of 25–67 μM. For electron microscopy, therefore, either the male- or female-derived enzyme could be used.

Disc Gel Electrophoresis

t-Butanol- and t-pentanol-containing buffers: Purified urate oxidase was dissolved in buffer A (tris, t-pentanol) or buffer B (tris, t-butanol) (see Methods). The pH of buffer A solutions was adjusted to 9.5, 10.0, or 10.6; buffer B was used only at pH 10.6. 50–150 μg of protein were applied to the gels and bidirectional electrophoresis carried out at room temperature for 0.17, 1.0, 2.0, 3.0, and 4.0 hours at each pH. The number of bands staining for protein varied from 1 at 50 μg protein to 4 at 150 μg protein, but all protein-staining bands also stained for urate oxidase activity.

TABLE I. Amino Acid Composition of Urate Oxidase Preparations Derived From Livers of Male and Female Rats (moles amino acid/moles half cystine*)

Amino acid	Male	Female
Lysine	10 (10.3)	10 (10.3)
Histidine	6 (5.9)	6 (5.9)
Arginine	5 (5.2)	5 (5.0)
Tyrosine[a]	5 (4.7)	5 (4.7)
Aspartic acid	12 (12.3)	12 (12.4)
Threonine[a]	8 (8.2)	8 (8.0)
Serine[a]	6 (6.1)	6 (6.2)
Glutamic acid	11 (11.2)	11 (11.2)
Proline	5 (4.8)	5 (4.9)
Glycine	7 (7.3)	7 (7.4)
Alanine[b]	4 (4.3)	5 (4.6)
Valine[b]	10 (9.9)	11 (10.6)
Methionine	2 (1.9)	2 (2.0)
Isoleucine[b]	8 (7.9)	7 (7.3)
Leucine	8 (8.2)	8 (8.0)
Phenylalanine	6 (5.8)	6 (5.8)
(Half cystine)	1	1

*Nearest integer; experimental figures in parentheses.
[a]Value obtained by extrapolation to zero time from 144 hours hydrolysis time.
[b]Value obtained by extrapolation through 144 hours hydrolysis time.

Acetonitrile-containing buffers: Buffer C (tris, acetonitrile, t-pentanol), was adjusted with acetic acid to pH's = 8.5, 9.0, or 9.5. 50 μg protein was applied to the gels and electrophorized bidirectionally for 0.17, 1.0, 2.0, and 3.0 hours at room temperature. At pH's = 8.5 and 9.0 extensive smearing resulted with no resolved bands. At pH = 9.5 the smearing was considerably reduced and several diffuse bands could be distinguished. Again all protein bands or smears stained for urate oxidase activity.

8 M Urea systems: An overload of 100 μg protein was applied to gels prepared with buffer D and electrophorized bidirectionally for 0.17, 1.0, 3.0, and 6.0 hours at room temperature. The gels were stained for protein but not enzymatic activity, which is absent in 8 M urea. Four anodic bands were observed.

Amino Acid Composition

In Table I are reported the amino acid compositions of urate oxidase preparations from the livers of male and female rats. The samples analyzed had been precipitated, redissolved, and reprecipitated as described under Methods. There were 9 moles of half cysteine residues per 1,000 moles of amino acid residues; columns 2 and 3 represent the moles of each amino acid relative to one-half cystein or per two methionines. It is evident that there are no differences in amino acid composition between the two preparations.

The sulfhydryl content of a sample of the female-derived preparation was estimated by the Elman method to be 1–2 per minimum molecular weight of 15,000.

The presence of tryptophan in the enzyme preparations was indicated by fluorescence observed with λ_{ex} = 280 nm, λ_{em} = 335 nm, although no attempt was made to quantitate these data.

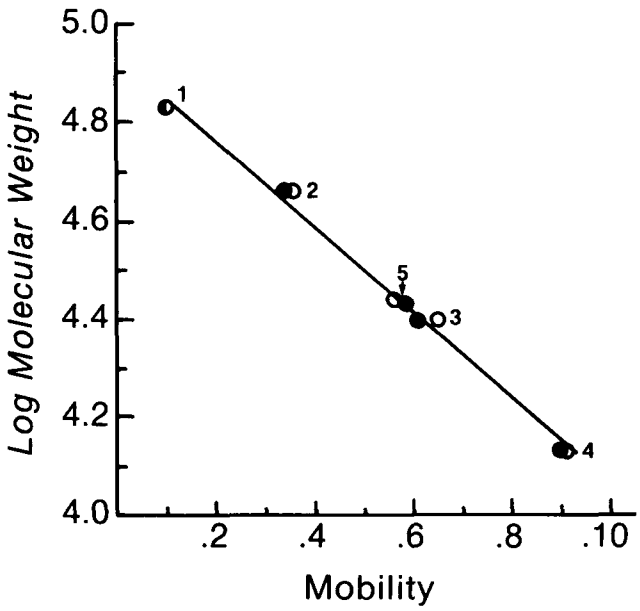


Fig. 2. Plot of log molecular weight of protein vs electrophoretic mobility: 1) bovine serum albumin, 2) ovalbumin, 3) chymotrypsinogen A, 4) cytochrome c, 5) urate oxidase preparation; ○) individual proteins, ●) mixture of proteins.

Estimation of Molecular Weight of Urate Oxidase Preparation From Female Rats

Cytochrome c, chymotrypsinogen A, ovalbumin, and bovine serum albumin were used as comparison proteins in an SDS-mercaptoethanol acrylamide gel system together with urate oxidase prepared from livers of female rats. Proteins were run individually on separate gels and as a mixture of proteins. In Fig. 2 are the results of one such experiment; from the plot of log molecular weight against mobility the molecular weight of the main urate oxidase protein band was determined to be 20,000–30,000 daltons. In Fig. 3, gel 5 indicates the high state of purity of the urate oxidase preparations; a minor band is evident.

Electron Microscopy

In Fig. 4 the appearance of rat liver peroxisomes in situ may be compared with that of the precipitated enzyme. The two views of the liver would seem to represent oblique and lateral sections through the nucleoid core. In the enzyme view an oblique and a lateral section also seem to have been achieved.

Effects of Starvation

Variation of specific urate oxidase activity with days of starvation is presented in Fig. 5; it is clear that activity falls after the first hours of inanition and steadily recovers through the fifth day. In Fig. 6 it is seen that starvation does affect protein content, but this would not alter the measured increase in enzyme specific activity. In Fig. 7 are typical electron microscopic views of liver sections corresponding to the tissues from which the enzymic data in Fig. 5 were obtained. It is apparent that the nucleoid core, present in the fed animal liver peroxisome, progressively diminishes during the early days of starvation, but reappears by the fifth day.

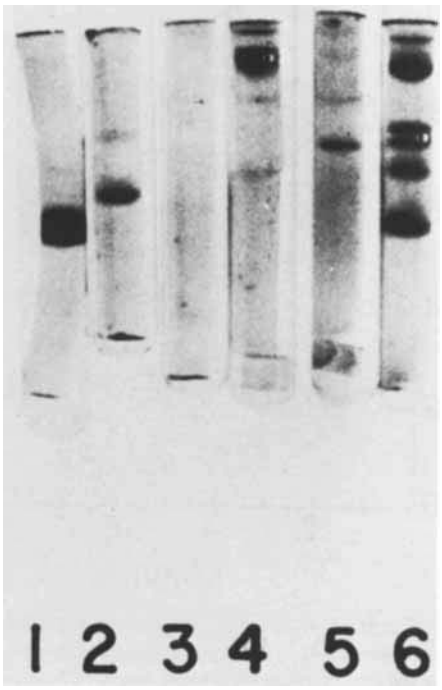


Fig. 3. SDS-acrylamide gel electrophoresis of a urate oxidase preparation and marker proteins: Gel 1, cytochrome c; Gel 2, chymotrypsinogen A; Gel 3, blank; Gel 4, bovine serum albumin; Gel 5, urate oxidase; Gel 6, mixture of all samples. Conditions are as described in the text.

The sections chosen in Fig. 7 were representative of the general appearance of the liver peroxisomes of animals from the various experimental populations and not artifacts created by tangential sectioning of nucleoids. To emphasize this point, larger fields of EM liver section were examined and the proportionate area of the peroxisome occupied by the nucleoid core was estimated as follows: Two axes, normal to each other, were centered in the nucleoid core, the length intercepted by the core was divided by that intercepted by the peroxisome, and the fractions for the two axes were averaged and reported as "nucleoid domain" (Table II, column 2). The data for one and two days of starvation show that significant changes have occurred in the nucleoid site, as also seen in Fig. 7. It was also clear that a large proportion of the peroxisomes had no cores, or only ill-defined structures (Table II, column 3).

In a separate series of experiments the activity of catalase, another liver peroxisomal enzyme, was also followed during a period of extended starvation; the results appear in Table III. Unlike urate oxidase activity, catalase activity expressed per mg of protein, is essentially unchanged by starvation. The changes in EM appearance noted in Fig. 7 above would correlate therefore with changes in urate oxidase, and not catalase activity.

Effects of Actinomycin D Injection on Responses of Urate Oxidase and Catalase to Starvation

Additional evidence that urate oxidase and catalase respond differently to starvation was supplied by the results in Table III. Actinomycin D injection had significantly greater effect on the urate oxidase activity, preventing the initial fall in activity during the first day of starvation, whereas the catalase activity was slightly increased. Injection of the anti-

biotic after twelve hours of starvation had no significant effects on the specific activity of either enzyme. This lack of effect of delayed injection was observed even for longer periods of starvation (Table III).

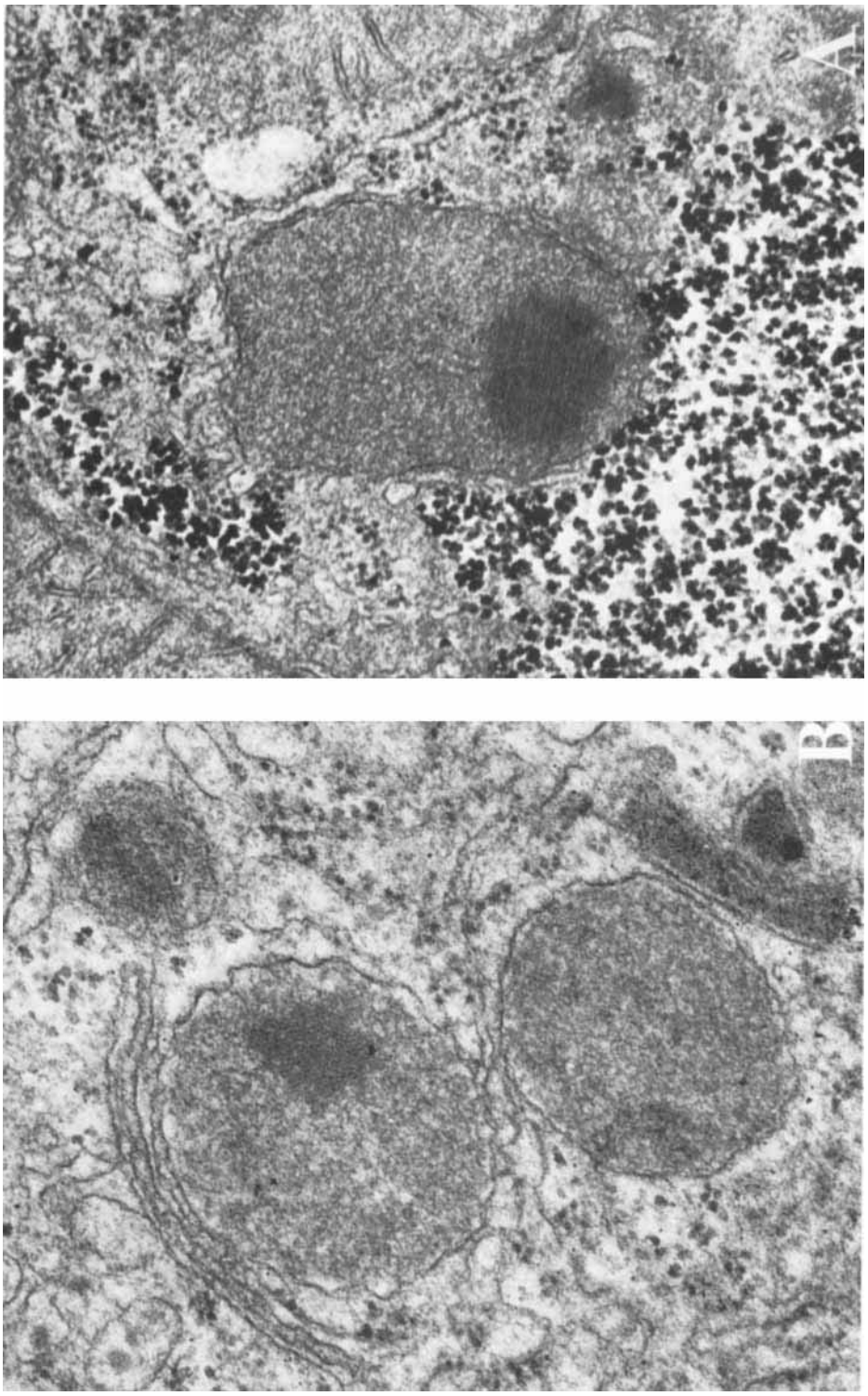
DISCUSSION

The rat liver urate oxidase preparation used in these studies was of sufficiently high purity, by several measures, that EM pictures thereof could reasonably be said to depict the precipitated enzyme itself, or the enzyme together with nonseparable moieties: The specific activity of the purified enzyme was equal to, or higher than, that of the most active product previously reported (5), ie, 13 units/mg protein compared to 11.7 units/mg protein. Acrylamide gel electrophoresis under all conditions used (acetonitrile-, pentanol-, or butanol-containing buffers and in the pH range 8.5–9.5) displayed bands all of which stained for protein and urate oxidase activity. In SDS-gels one major protein-staining band and a minor band (representing a trace of protein) appeared. The amino acid compositions of enzymes prepared from livers of either male or female rats were identical; data showing identity of primary structures would be preferable, but in their absence compositional identity suggests consistency in the product obtained. The molecular weight suggested by SDS-gel electrophoresis was comparable to that reported by others (5).

The electron microscopic appearance of these preparations suggests that longitudinal and oblique sections were made through a polytubular structure (Fig. 4c). These are reminiscent of the EM appearance of similar sections through in situ peroxisomal nucleoids (Fig. 4a,b).

It might still have been possible that the precipitated purified enzyme assumes, fortuitously, an EM appearance similar to that of the peroxisomal core but does not represent the in vivo situation. The results from the starvation experiments demonstrate parallel initial decreases and then increases in the total and specific liver urate oxidase activity and in the intensity and occurrence of the peroxisomal nucleoid core in EM pictures of liver sections taken from the same organs. The specificity of the starvation effects for urate oxidase is indicated by the markedly different effect, or lack thereof, on the specific activity of catalase, another peroxisomal enzyme. The observed biphasic response of urate oxidase activity to starvation reaffirmed a previous report (11). Constancy of catalase activity under similar conditions was observed by Rechcigl and Price (21). Differential control of turnover of catalase and of urate oxidase in a different species, the mouse, has been reported (22). The differences in responses of the measured activity of these two enzymes to starvation is further emphasized by the different effects observed on their activities in response to actinomycin D injection. For example, catalase activity increased 29% when actinomycin D was injected at the start of the period of inanition, whereas urate oxidase activity increased 63% above uninjected controls; when actinomycin D injection was delayed, differential effects on the two enzyme activities were still observed (Table III). These data are consistent with the suggestion that rat liver urate oxidase and catalase enzyme levels respond quite differently to starvation under a variety of conditions – and that the starvation-associated changes in peroxisomal EM appearance are urate oxidase-associated.

This report therefore presents evidence from concerted experiments involving electron microscopy, enzyme purification, and inanition effects in a single species, the rat. In the liver of this animal intimate association of the peroxisomal nucleoid and urate oxidase is established. The mechanism for the formation of such a supramolecular structure in vivo remains to be determined.


A
B

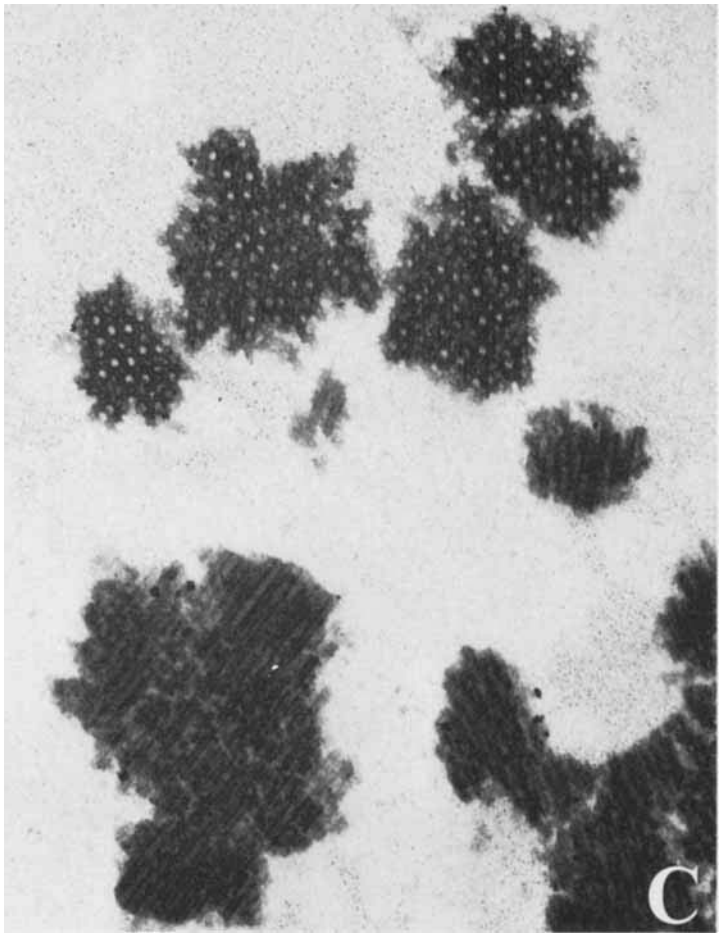


Fig. 4. Electron microscopic comparison of rat liver peroxisomes and precipitated purified urate oxidase (samples prepared as described in text): a) section through peroxisome in parallel with long axis of possible polytubular structure of nucleoid, b) section through peroxisome to give an oblique slice of possible polytubular structure of nucleoid, c) section through precipitated enzyme preparation (magnification: a and b 63,270 ×; c 75,810 ×).

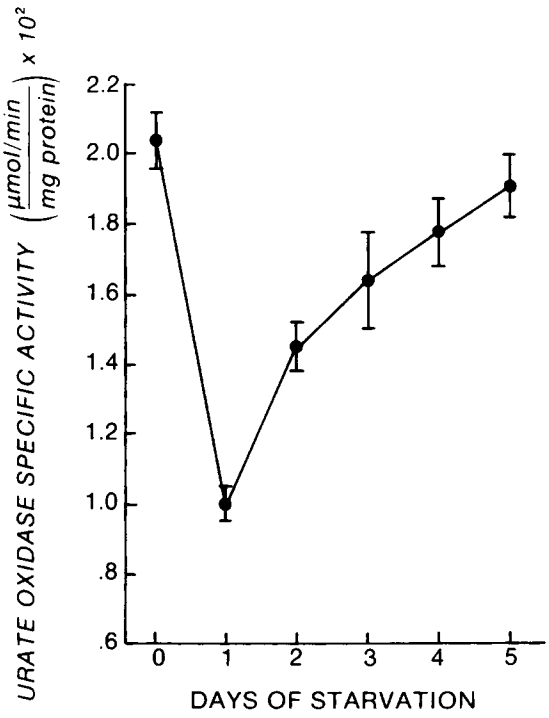


Fig. 5. Change in specific activity of rat liver urate oxidase during a five-day starvation period. Each point is the average of triplet determinations with two animals. Bars indicate standard deviation at 95% confidence interval using t-test calculations.

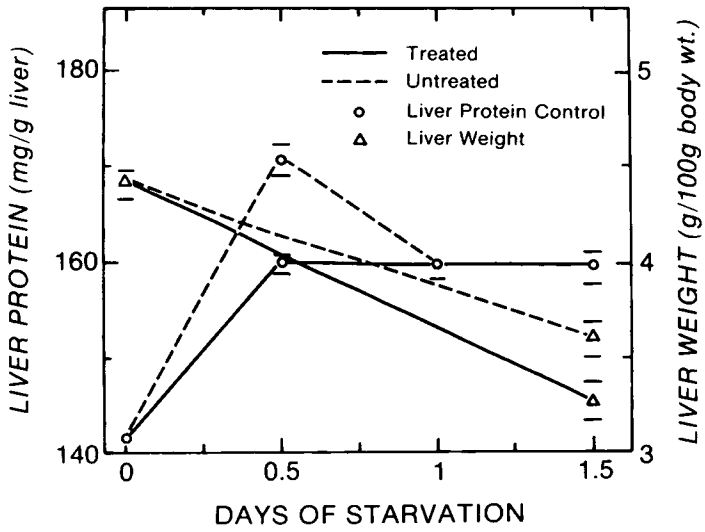


Fig. 6. The effect of starvation and actinomycin D on liver protein content and liver weight: △) liver weight, ○) liver protein content.

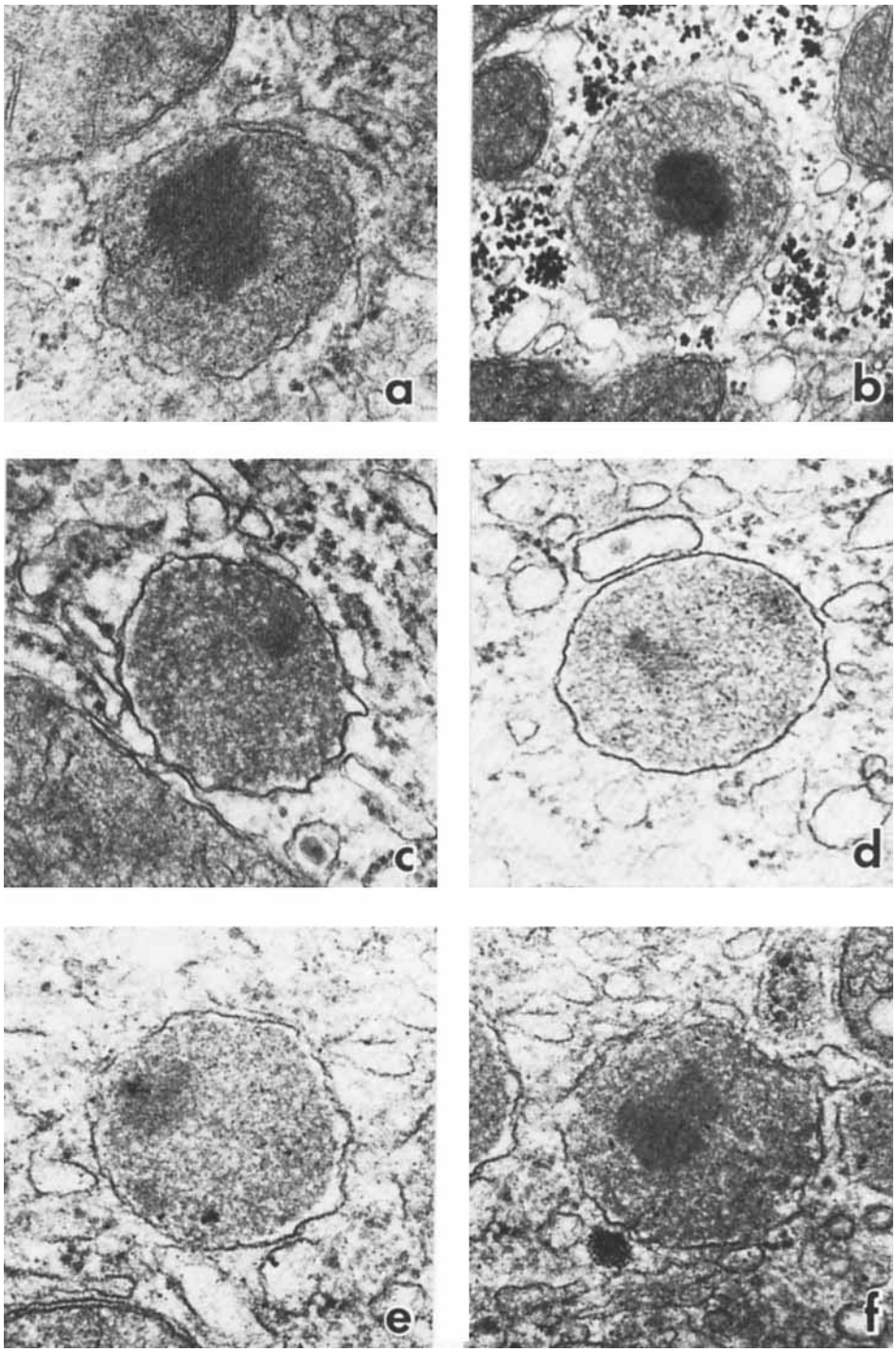


Fig. 7. Electron microscopic appearance of rat liver peroxisomes at various times (hours) of starvation. For each experimental period a representative microscopic field was chosen from liver sections from each of two rats. Plate 1: a,b 0 hours; c,d 24 hours; e,f 48 hours.

Figure 7 (cont'd)

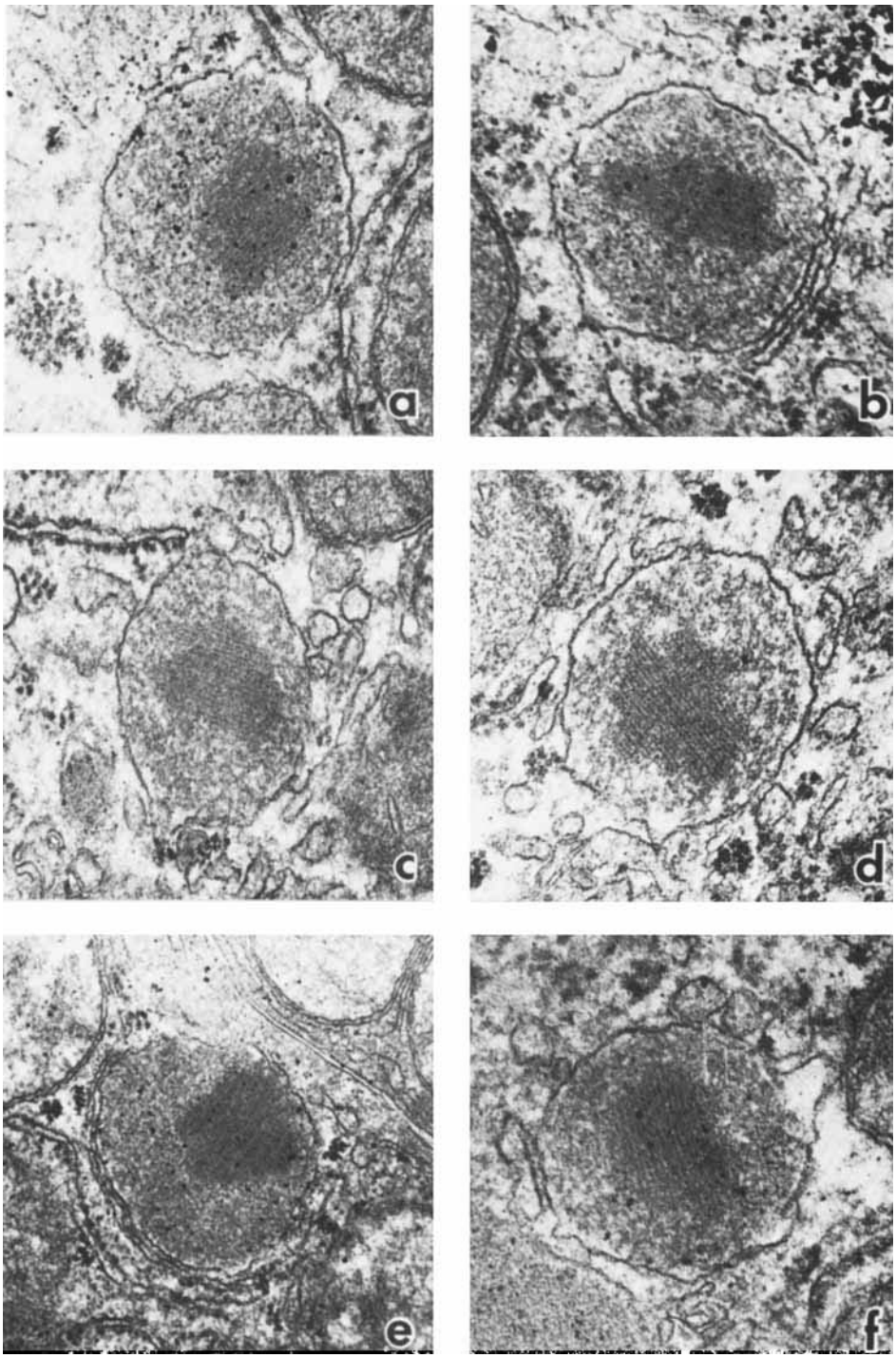


Fig. 7. Plate 2: a, b 72 hours; c, d 96 hours; e, f 120 hours.

TABLE II. Effect of Starvation on Peroxisomal Nucleoids

Days of starvation	Nucleoid domain (SD)	Percentage peroxisomes with ill-formed or absent nucleoids
0 (control)	0.43 (0.75)	10
1	0.30[a] (0.13)	65
2	0.34[a] (0.13)	50
3	0.42[b] (0.21)	0
4	0.44[b] (0.089)	6
5	0.51[c] (0.11)	0

*Data average 120 readings.
[a]Significantly different from control at 0.01% level by t-test.
[b]Not significantly different from control.
[c]Significantly different from control at 3% level by t-test.

TABLE III. Effect of Actinomycin D on Catalase and Urate Oxidase Activity During Starvation

	Experimental conditions			Catalase		Urate oxidase	
Expt	Initial actinomycin D injection time (hr)	Duration of inanition (hr)	Actinomycin D dose per injection (mg/kg)	Activity ± SE (units)[a]	% Change from untreated control	Activity ± SE (units)[b]	% Change from untreated control
Control	0	0	0	482 ± 13 (4)[c]		0.29 ± 0.01 (12)[c]	
I	0	12	0	471 ± 15 (3)	− 2	0.17 ± 0.01 (3)	
			1.2	607 ± 25 (3)	+ 29	0.28 ± 0.00 (3)	+ 63
			0.6	609 ± 16 (3)	+ 29	0.26 ± 0.00 (3)	+ 54
II	0	24	0	578 ± 15 (4)		0.19 ± 0.02 (4)	
			0.6	535 ± 11 (3)	− 7	0.27 ± 0.00 (3)	+ 39
			0.3	552 ± 34 (3)	− 4	0.24 ± 0.01 (3)	+ 20
	12	24	1.2	536 ± 42 (3)	− 7	0.18 ± 0.00 (3)	− 6
			0.6	531 ± 22 (3)	− 8	0.20 ± 0.01 (3)	+ 2
III	12	36	0	420 ± 10 (4)		0.21 ± 0.01 (4)	
			0.6	434 ± 14 (3)	+ 3	0.24 ± 0.01 (3)	+ 14
			0.3	462 ± 38 (3)	+ 10	0.26 ± 0.02 (3)	+ 27
IV	24	36	0	394 ± 31 (2)		0.19 ± 0.02 (2)	
			1.2	409 ± 60 (2)	+ 4	0.20 ± 0.03 (2)	+ 3
V	24	48	0	591 ± 58 (2)		0.24 ± 0.01 (2)	
			0.6	561 ± 34 (2)	− 6	0.31 ± 0.01 (2)	+ 32

[a]Unit of catalase activity: 1 μmole H_2O_2 destroyed/3 min/mg protein.
[b]Unit of urate oxidase activity: 1 μmole urate destroyed/30 min/mg protein. Determined colorimetrically.
[c]The number of animals in parentheses.

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