0022-1554/83/$3.00
The Journal of Histochemistry and Cytochemistry

Vol. 31, No. 10, pp. 1183–1189, 1983
Printed in U.S.A.

Quantitative Immunohistochemistry:

A Comparison of Microdensitometric Analysis of Unlabeled Antibody Peroxidase–Antiperoxidase Staining and of Microfluorometric Analysis of Indirect Fluorescent Antibody Staining for Nicotinamide Adenosine Dinucleotide Phosphate (NADPH)–Cytochrome *c* (P-450) Reductase in Rat Liver[1]

MARTYN T. SMITH,[2] JAN A. REDICK, and JEFFREY BARON[3]

Toxicology Unit, Department of Pharmacology (M.T.S.), The School of Pharmacy, University of London, London WC1N 1AX, England and The Toxicology Center, Department of Pharmacology (J.A.R.; J.B.), College of Medicine, The University of Iowa, Iowa City, Iowa 52242

Received for publication August 24, 1982 and in revised form February 9, 1983; accepted February 19, 1983 (OA 82-321)

The intralobular distribution of nicotinamide adenine dinucleotide phosphate (NADPH)–cytochrome *c* (P-450) reductase (NADPH:ferricytochrome oxidoreductase, EC 1.6.2.4) in rat liver has been investigated by means of two quantitative immunohistochemical techniques: microdensitometric quantitation of unlabeled antibody peroxidase–antiperoxidase staining and microfluorometric analysis of indirect fluorescent antibody staining. Utilizing sheep antiserum elicited against NADPH–cytochrome *c* (P-450) reductase that had been isolated and purified to apparent homogeneity from rat liver microsomes, the reductase was detected within hepatocytes throughout the liver. However, differences in the intensity of staining of hepatocytes within different regions of the liver lobule were readily apparent after completion of both immunohistochemical staining procedures. These visual findings were verified by microdensitometric and microfluorometric analyses of immunohistochemical staining, both of which revealed that approximately the same degree of staining for NADPH–cytochrome *c* (P-450) reductase was produced within the centrilobular and midzonal regions of the liver lobule, whereas periportal hepatocytes were stained with significantly less intensity. These results demonstrate that the application of either microdensitometry in conjunction with unlabeled antibody peroxidase–antiperoxidase staining or microfluorometry after indirect fluorescent antibody staining can be used to quantitatively determine the intratissue distributions of antigens.

KEY WORDS: NADPH–cytochrome *c* (P-450) reductase; Rat liver; Immunohistochemical localization; Quantitative immunohistochemistry; Microdensitometry; Microfluorometry.

Introduction

Immunohistochemical techniques are now widely used to determine the localizations of enzymes and other substances of biological importance within tissues and cells. Studies employing these techniques, however, are usually of a qualitative nature, with little or no attempt being made to obtain quantitative data. Since the ability to quantitate cellular antigens would provide a great deal of important biological information, methods for the quantitation of immunohistochemical findings should be sought.

This laboratory has employed both unlabeled antibody peroxidase–antiperoxidase and indirect fluorescent antibody staining techniques to investigate the intrahepatic localizations and distributions of several enzymes, including nicotinamide adenine dinucleotide phosphate (NADPH)–cytochrome *c* (P-450) reductase (1,10,16,17), cytochromes P-450 (2,3,10), and epoxide hydrolase (7,10), that participate in the bioactivation and detoxification of endogenous and exogenous substances. In these studies, microfluorometric determinations of the intensities of fluorescence emitted from hepatocytes after indirect fluorescent antibody staining provided semiquantitative data

[1]Supported by a grant from the Medical Research Council of Great Britain and by United States Public Health Service Grants GM 12675 and CA 30140.

[2]Present address: Department of Biomedical and Environmental Health Sciences, School of Public Health, University of California, Berkeley, California 94720.

[3]To whom reprint requests should be addressed.

regarding the relative extents of specific antibody binding within centrilobular, midzonal, and periportal regions of the liver lobule.

In the present study, scanning and integrating microdensitometry was employed to obtain quantitative data from liver sections stained for NADPH–cytochrome *c* (P-450) reductase utilizing the unlabeled antibody peroxidase–antiperoxidase staining method. Results of microdensitometric analyses were directly compared to microfluorometric measurements of indirect fluorescent antibody staining for the reductase. In addition, microdensitometric measurements within individual hepatocytes allowed for the determination of the exact intralobular distribution of NADPH–cytochrome *c* (P-450) reductase within rat liver.

Materials and Methods

Purification of NADPH–cytochrome c (P-450) reductase and production of antibody. NADPH–cytochrome *c* (P-450) reductase, solubilized by tryptic digestion of rat liver microsomes, was purified to apparent homogeneity employing minor modifications (18) of the method described by Omura and Takesue (9). Sheep antiserum to the reductase was obtained as described previously (18), and whole sheep anti-reductase and normal (nonimmune) sera were employed in the immunohistochemical staining procedures. Normal sheep serum, the soluble sheep peroxidase–antiperoxidase complex, rabbit antiserum to sheep immunoglobulin (Ig) G, and fluorescein isothiocyanate (FITC) conjugates of IgG prepared from rabbit antiserum to sheep IgG were obtained from Miles Laboratories, Inc.

Immunohistochemical staining procedures. Male albino Holtzman rats, weighing 180–230 g, were used. They were allowed food and water ad libitum. Rats were killed by decapitation, the livers were immediately excised, and the median lobe of each was cut into blocks approximately 2 mm in thickness. The liver blocks were fixed at 4°C for a total period of 4 hr by immersion in several changes of a solution containing 0.35% (w/v) parabenzoquinone (Polysciences, Inc.) and 0.02 M $CaCl_2$ in 0.2 M sodium cacodylate buffer, pH 7.4. The fixed blocks were then dehydrated, cleared, embedded in paraffin, and serial sections 7 μm in thickness were prepared, placed on albumin-coated slides, and dried overnight at 37°C. Prior to performance of the immunohistochemical staining procedures, the sections were dewaxed in xylene and rehydrated in graded methanols.

The immunohistochemical localization of NADPH–cytochrome *c* (P-450) reductase in rat liver was accomplished employing minor modifications of the unlabeled antibody peroxidase–antiperoxidase (1–3,7,15–18) and the indirect fluorescent antibody (2,3,7,16–18) staining techniques. In the unlabeled antibody peroxidase–antiperoxidase method, after the endogenous peroxidase activity of the tissue had been blocked (1), the sections were exposed for 40 min to 10% (v/v) dimethyl sulfoxide prior to completion of the staining protocol (1). We have found that dimethyl sulfoxide enhances the uniform penetration of antibodies into tissue sections and yields much more reproducible immunohistochemical staining. In the indirect fluorescent antibody technique, the sections were similarly exposed to dimethyl sulfoxide, and after exposure to either sheep anti-reductase serum or normal sheep serum (both of which had been diluted 1:500 with 0.05 M Tris-HCl buffer, pH 7.75, containing 0.154 M NaCl), they were exposed for 1 hr at 37°C to FITC-conjugated IgG of rabbit antiserum to sheep IgG that had been diluted 1:100 with Tris-buffered saline. The sections were then examined by incident-light fluorescence microscopy using a modified Leitz Orthoplan microscope (19) with a 150-W Osram xenon lamp and a Leitz Ploemopak 2.1 fluorescence illuminator containing a K2 filter block (excitation, 470–490 nm; RKP 510 beam-splitting mirror; LP 515 suppression filter) (2).

Microfluorometric and microdensitometric quantitation of immunohistochemical staining. The fluorescence emitted from 2.5 × 2.5 μm areas within hepatocytes was transmitted through a Leitz microfluorometric attachment to a Schoeffel GM 100 monochromator, and fluorescence emission at 525 nm was detected by an EMI 9658A photomultiplier tube. The signal from the photomultiplier tube was then amplified by a Schoeffel M 460 photometer. Because fluorescence intensity should be linearly related to the concentrations of FITC and antigen, and because absorbance is a linear function of sample concentrations, whereas transmittance is a logarithmic function, the output from the photometer was fed into a log–linear converter designed and built by the Bioengineering Facility of The University of Iowa College of Medicine that converts the fluorescence emission intensity (transmitted light) into an absorbance value. The output from the log–linear converter was then displayed on an Axiom EX-801P digital printer (Axiom Corp.). Since absorbance decreases as the intensity of emitted fluorescence increases, the absorbance value was subtracted from 1, and the results are expressed in terms of 1 − absorbance (× 100). In this manner, a positive, linear relationship is obtained between the intensities of indirect fluorescent antibody staining and the microfluorometric measurements. A uranyl glass standard attached to a Plezy adaptor was employed to calibrate the microfluorometric apparatus. By doing this, less than 5% variability is found in microfluorometric measurements obtained from within a given section and from within corresponding hepatocytes in multiple liver sections.

Osmium black, the product formed by the osmication of oxidized 3,3′-diaminobenzidine in the unlabeled antibody peroxidase–antiperoxidase staining technique (11), was quantitated using a Vickers M85 scanning and integrating microdensitometer. The use of this instrument to overcome problems associated with the measurement of heterogeneously distributed reaction products in tissue sections has been discussed in detail by Chayen (4). The microdensitometer was interfaced to a CBM PET microcomputer to facilitate data handling and statistical analysis (14).

As seen from the data presented in Figure 1, osmium black exhibits a relatively broad absorption band having a maximum at 430 nm. The absorbance of osmium black at 430 nm was determined in tissue sections using a × 40 objective, spot size 1 (being 0.5 μm in diameter in the optical plane of the specimen), and a field size of 5 μm diameter. Using these conditions, it was possible to obtain microdensitometric measurements from within the cytoplasm of individual hepatocytes. The results of microdensitometric analyses of unlabeled antibody peroxidase–antiperoxidase staining are expressed in terms of integrated absorbance units. The Vickers microdensitometer was calibrated before use by employing a series of filters of known absolute absorbance (4).

For quantitative analyses of immunohistochemical staining for NADPH–cytochrome *c* (P-450) reductase within liver, two pairs of serial sections were prepared from each of 6 livers. One section of each pair was exposed to sheep anti-reductase serum, and the other section was exposed to normal sheep serum. One pair of sections was then stained employing the indirect fluorescent antibody method, while the second pair was stained using the unlabeled antibody peroxidase–antiperoxidase technique. In each tissue section, microfluorometric or microdensitometric measurements were taken from 15 hepatocytes lying within the first 5 cell layers surrounding central veins of less than 40 μm diameter (centrilobular hepatocytes), from 15 hepatocytes lying within 3 cell layers on either side of midlines between central veins and portal tracts (midzonal hepatocytes), and from 15 hepatocytes lying within the first 5 cell layers surrounding portal tracts of

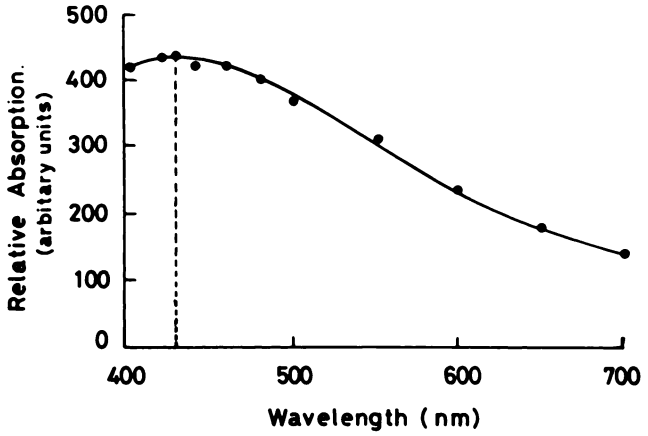

Figure 1. Absorption spectrum of osmium black determined in sections of rat liver by means of scanning and integrating microdensitometry. The vertical dashed line is at 430 nm.

less than 40 μm diameter (periportal hepatocytes). To calculate the fluorescence or absorbance due to anti-reductase binding to centrilobular, midzonal, and periportal hepatocytes, the mean microfluorometric or microdensitometric value obtained from within each region in the section exposed to normal sheep serum was subtracted from each individual measurement obtained from within the corresponding region in the serial sections exposed to sheep antiserum to NADPH–cytochrome *c* (P-450) reductase. Mean ± SEM values reported in Tables 1 and 2 were calculated from the values obtained from each liver.

Statistical analysis. The results of microdensitometric analyses were compared to those obtained by microfluorometry by estimating the pooled variance and performing one-way analyses of variance on the data obtained with each method. Where appropriate, the group Student's *t*-test was also used. The level of significance was taken as 5% ($p < 0.05$). The coefficient of variation of the data for each region was calculated for both methods in order to compare the relative variability of the data produced. The use of the coefficient of variation as an indicator of the degree of variability eliminates the need to take into account the fact that the microdensitometric values for anti-reductase binding were numerically greater than the microfluorometric values.

Results

Immunohistochemical Localization of NADPH–Cytochrome c (P-450) Reductase in Rat Liver

As reported previously by this laboratory (1,10,16,17), when sections of rat liver were exposed to sheep antiserum to rat hepatic microsomal NADPH–cytochrome *c* (P-450) reductase in both the unlabeled antibody peroxidase–antiperoxidase and the indirect fluorescent antibody staining protocols, hepatocytes throughout the liver were stained for the enzyme (Figure 2A,C). Immunohistochemical staining for the reductase was not apparent within hepatocytes in sections that had been exposed to normal sheep serum (Figure 2B,D). It is readily apparent from the photomicrographs in panels A and C of Figure 2, however, that periportal hepatocytes are much less intensely stained for the reductase than are either centrilobular or midzonal hepatocytes. This observation indicates that periportal hepatocytes contain less enzyme than do those cells lying within the centrilobular and midzonal regions of the liver lobule. To quantitatively investigate this possibility, microfluorometric and microdensitometric analyses were conducted to determine the extent of anti-reductase binding to hepatocytes lying within the three regions of the liver lobule.

Microfluorometric Quantitation of Indirect Fluorescent Antibody Staining

The results of microfluorometric analyses of indirect fluorescent antibody staining of centrilobular, midzonal, and periportal hepatocytes are shown in Table 1. The fluorescence emitted from within cells in sections exposed to normal sheep serum is due to both nonspecific tissue autofluorescence and nonspecific binding of FITC-conjugated IgG. In all instances, cells in sections exposed to sheep anti-reductase serum emit fluorescence with significantly ($p < 0.01$) greater intensity. In an earlier study (16), removal of the antibody from the antiserum by adsorption with purified rat hepatic microsomal NADPH–cytochrome *c* (P-450) reductase was shown to cause the intensity of emitted fluorescence to decrease to that measured within cells in sections exposed to normal sheep serum. The data summarized in Table 1 indicate that the anti-reductase bound equally to centrilobular and midzonal hepatocytes, while significantly less anti-reductase bound to periportal hepatocytes. These microfluorometric findings are thus consistent with visual observations of indirect fluorescent antibody staining for the reductase within the liver lobule (Figure 1C) and are also in agreement with those reported previously (10,16,17).

Microdensitometric Quantitation of Unlabeled Antibody Peroxidase–Antiperoxidase Staining

The amount of osmium black deposited within the cytoplasm of centrilobular, midzonal, and periportal hepatocytes after unlabeled antibody peroxidase–antiperoxidase staining was determined microdensitometrically (Table 2). The mean absorbance of sections that had not been subjected to staining was approximately 0.13 within all regions of the lobule (data not shown), whereas sections exposed to normal sheep serum in the staining protocol had an overall mean absorbance of approximately 0.28 (Table 2). The absorbance values determined after exposure to normal sheep serum, therefore, represent the amount of absorption due to both light scattering and nonspecific staining. As seen from the data presented in Table 2, the absorbance of cells in sections exposed to sheep anti-reductase serum was in all cases significantly ($p < 0.01$) greater than that of cells in sections exposed to normal sheep serum. Calculation of the absorbance due to anti-reductase binding revealed that the antibody bound to similar extents within centrilobular and midzonal regions of the liver lobule, whereas significantly less binding occurred within periportal regions. After the anti-reductase had been removed from the antiserum by adsorption with the purified enzyme, anti-reductase binding was decreased by approximately 90%: the

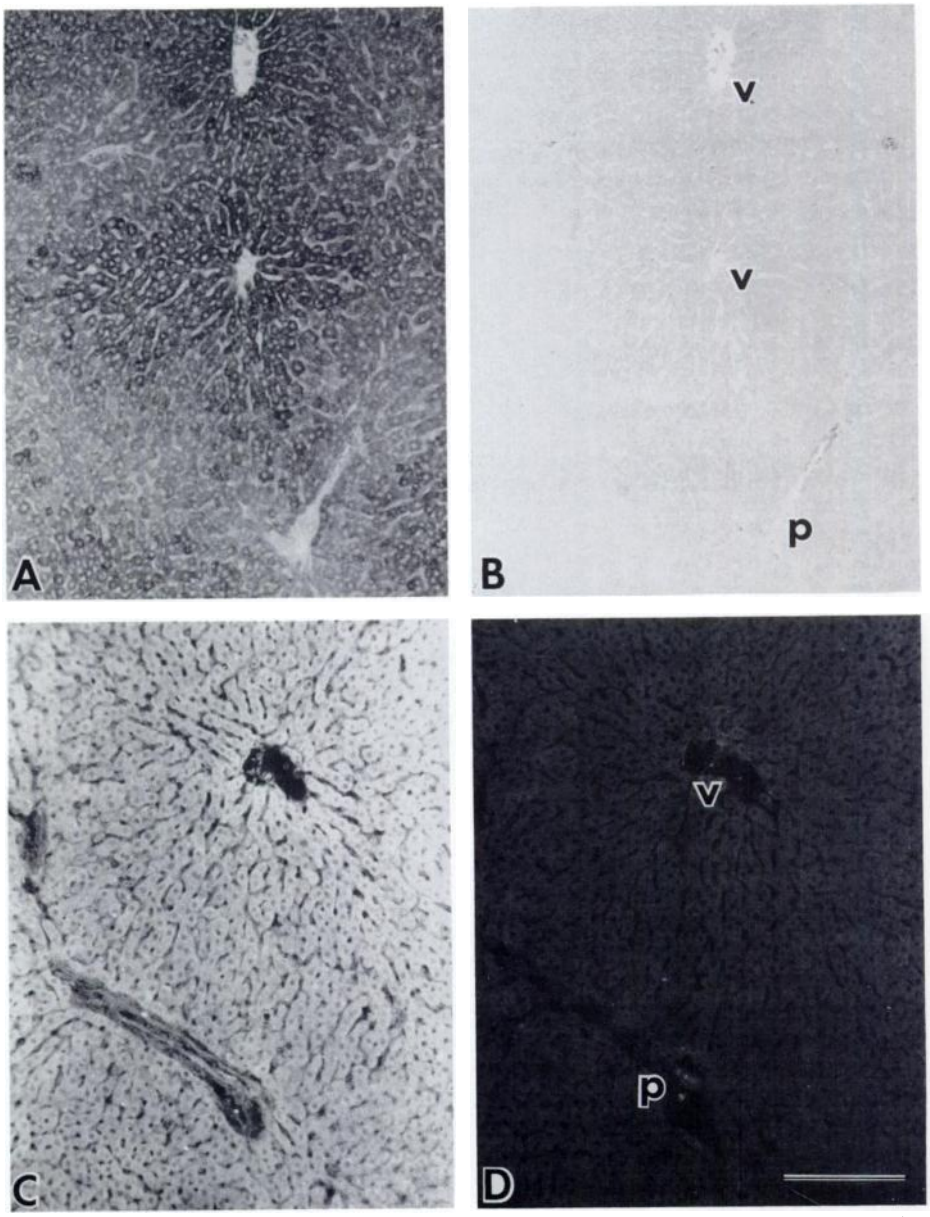

Figure 2. Immunohistochemical localization of NADPH–cytochrome *c* (P-450) reductase within rat liver. The photomicrographs show areas in 7-μm-thick sections prepared from the liver of a male rat. (**A**) Section exposed to sheep anti-reductase serum in the unlabeled antibody peroxidase–antiperoxidase staining protocol. (**B**) Serial section exposed to normal sheep serum. (**C**) Section exposed to sheep anti-reductase serum in the indirect fluorescent antibody staining protocol. (**D**) Serial section exposed to normal sheep serum. The sheep anti-reductase serum and normal serum had each been diluted 1:500 with 0.05 M Tris-HCl buffer, pH 7.75, containing 0.154 M NaCl. Central veins (V) and portal tracts (P) are indicated in **B** and **D**. Original magnification × 165. Bar = 200 μm.

mean ± SEM absorbance at 430 nm of centrilobular, midzonal, and periportal hepatocytes in sections exposed to adsorbed anti-reductase serum was 0.37 ± 0.02, 0.34 ± 0.02, and 0.31 ± 0.02, respectively. These microdensitometric findings are thus in agreement with those obtained from microfluorometric analyses and further demonstrate that NADPH–cytochrome *c* (P-450) reductase is not uniformly distributed within the liver lobule.

Comparison of Microfluorometric and Microdensitometric Determinations

Results of microdensitometric analyses of unlabeled antibody peroxidase–antiperoxidase staining for NADPH–cytochrome *c* (P-450) reductase within the liver lobule were directly compared to those obtained by microfluorometry following indirect fluorescent antibody staining by means of one-way analyses of variance. The results of these analyses demonstrated that there was no significant difference ($p > 0.05$) in the extent of anti-reductase binding to centrilobular and to midzonal hepatocytes as determined by microfluorometry and microdensitometry, whereas both methods showed that significantly less ($p < 0.001$) anti-reductase bound to periportal hepatocytes (Tables 1 and 2). Thus, the two methods for the quantitation of immunohistochemical staining yield very similar results. The coefficients of variation of the values determined with the two methods for the extents of antireductase binding within centrilobular, midzonal, and periportal regions of the liver

Table 1. *Microfluorometric measurements of the intensity of indirect fluorescent antibody staining for NADPH–cytochrome c (P-450) reductase within different regions of the liver lobule*[a]

Region	Emitted fluorescence after exposure to sheep anti-reductase serum	Emitted fluorescence after exposure to normal sheep serum	Fluorescence due to anti-reductase binding
Centrilobular	69.1 ± 0.3	43.9 ± 0.2	25.2 ± 0.4[b]
Midzonal	68.3 ± 0.4	43.4 ± 0.3	24.9 ± 0.6[b]
Periportal	55.7 ± 0.4	43.7 ± 0.5	12.0 ± 0.5[c]

[a]The values are given as the mean ± S.E.M. of six rats and are expressed in terms of 1 − absorbance (× 100).
[b]Values are not significantly different from each other, $p > 0.05$.
[c]Value is significantly lower than corresponding values from the centrilobular and midzonal regions, $p < 0.001$.

lobule were found to be 0.038, 0.058, and 0.092, respectively, by microfluorometry and 0.145, 0.120, and 0.144, respectively, by microdensitometry. From these findings, it can be concluded that there was an average of 2 to 3 times more variation in the microdensitometric values than in the microfluorometric values. This may, in part, reflect the potentially greater accuracy of microfluorometry over microdensitometry (8).

Determination of the Intralobular Distribution Curve of NADPH–Cytochrome c (P-450) Reductase in Rat Liver by Microdensitometry

The intralobular distribution of the reductase was further investigated by microdensitometrically determining the absorbance at 430 nm from within 15 to 20 hepatocytes situated along at least four imaginary straight lines between selected central veins and portal tracts in each tissue section. Subtraction of the absorbance due to light scattering and nonspecific staining, that is, values from sections exposed to normal serum, from the absorbance measured within corresponding cells in serial sections exposed to anti-reductase serum generated the intralobular distribution curve for the binding of the anti-reductase within the liver lobule. By pooling the values obtained from 4 rats, it was possible to produce the mean intralobular distribution curve of NADPH–cytochrome *c* (P-450) reductase in rat liver shown in Figure 3. From these data, it can be seen that hepatocytes adjacent to the central vein contain approximately twice as much reductase as do those adjacent to the portal tract. While the content of the reductase was found to be fairly constant within both centrilobular and periportal hepatocytes, variability in enzyme content was noted among midzonal cells, especially when the content within those cells adjacent to the centrilobular region was compared to that within midzonal cells that are adjacent to the periportal region.

Discussion

We previously reported (1,10,16,17) that an inhibitory antibody directed against rat hepatic microsomal NADPH–cytochrome *c* (P-450) reductase could be used to determine both the cellular localization and the intralobular distribution of the enzyme within rat liver. In these investigations, visual observations of unlabeled antibody peroxidase–antiperoxidase and indirect fluorescent antibody staining revealed that, although present within all hepatic parenchymal cells, NADPH–cytochrome *c* (P-450) reductase was not uniformly distributed across the liver lobule. Microfluorometric measurements of the intensity of indirect fluorescent antibody staining provided semiquantitative data, in terms of relative fluorescence units, for similarities and differences in the extents of anti-reductase binding to hepatocytes within the different regions of the liver lobule.

Quantitative data has now been obtained from sections of rat liver stained for NADPH–cytochrome *c* (P-450) reductase using the unlabeled antibody peroxidase–antiperoxidase method. In this immunohistochemical staining technique, after the anti-reductase has interacted with and bound to the enzyme present in the tissue section, it is coupled to a peroxidase–antiperoxidase complex. The subsequent exposure of this complex to 3,3′-diaminobenzidine and H_2O_2 results in the precipitation of oxidized diaminobenzidine that, when chelated with OsO_4,

Table 2. *Microdensitometric measurements of the intensity of unlabeled antibody peroxidase–antiperoxidase staining for NADPH–cytochrome c (P-450) reductase within different regions of the liver lobule*[a]

Region	Absorbance after exposure to sheep anti-reductase serum	Absorbance after exposure to normal sheep serum	Absorbance due to anti-reductase binding
Centrilobular	81.9 ± 1.1	30.0 ± 2.6	51.9 ± 3.1[b]
Midzonal	75.7 ± 1.8	28.0 ± 2.2	47.7 ± 2.3[b]
Periportal	57.6 ± 1.6	26.0 ± 1.5	31.6 ± 1.9[c]

[a]The values are given as the mean ± SEM of six rats and are expressed as integrated absorbance units (× 100).
[b]Values are not significantly different from each other, $p > 0.05$.
[c]Value is significantly lower than corresponding values from the centrilobular and midzonal regions, $p < 0.001$.

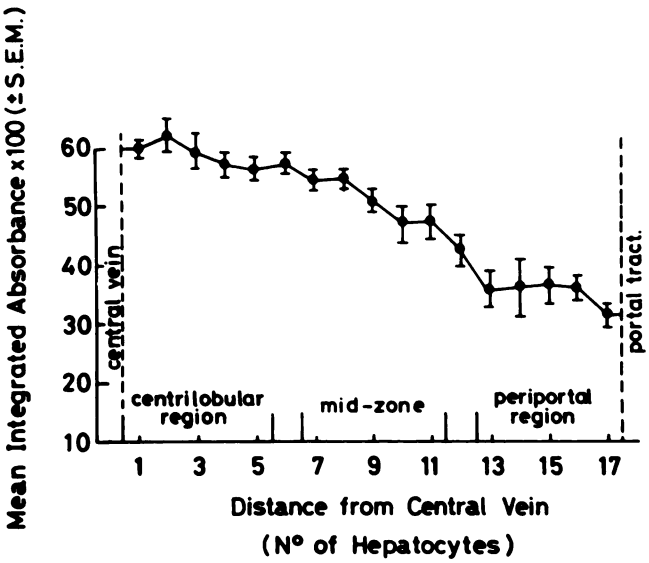

Figure 3. Intralobular distribution curve of NADPH–cytochrome *c* (P-450) reductase in rat liver determined by microdensitometry after unlabeled antibody peroxidase–antiperoxidase staining. The values are given as the mean ± SEM of at least 16 determinations made using sections of livers prepared from 4 rats.

produces a brown particulate deposit (osmium black) at the site of the antigen–antibody complex (11). In the present investigation, the amount of osmium black formed within hepatocytes was quantitated using a scanning and integrating microdensitometer. The microdensitometric results were then directly compared to those obtained by microfluorometric quantitation of indirect fluorescent antibody staining for the reductase within the three regions of the liver lobule.

Although microfluorometric quantitation is theoretically more precise than microdensitometry (8), microdensitometric quantitation of unlabeled antibody peroxidase–antiperoxidase staining has a number of distinct advantages to offer over the microfluorometric quantitation of indirect fluorescent antibody staining. First of all, the unlabeled antibody peroxidase–antiperoxidase method produces a superior resolution of intracellular staining than does the indirect fluorescent antibody staining method. Secondly, peroxidase–antiperoxidase staining is stable, whereas fluorescent staining is not and, thus, is not subject to errors such as those resulting from the fading of the stain. Finally, microdensitometric measurements can be expressed as absolute units of absorbance, so that if the molar absorptivity of the reaction product is known, the values obtained can be converted into conventional biochemical units.

Statistical analysis of the results obtained in this quantitative immunohistochemical investigation revealed that microdensitometric measurements of unlabeled antibody peroxidase–antiperoxidase staining and microfluorometric quantitation of indirect fluorescent antibody staining for NADPH–cytochrome *c* (P-450) reductase yielded very similar results. Both methods showed that approximately the same degree of staining for the reductase was produced within the centrilobular and midzonal regions of the liver lobule, whereas significantly less staining occurred within the periportal regions. Moreover, a high degree of statistical correlation was found between the results obtained with the two quantitative methods.

From these observations, it can be concluded that the content of NADPH–cytochrome *c* (P-450) reductase is 1.6 to 2.0 times greater in centrilobular hepatocytes than in periportal hepatocytes. This distribution is, thus, very similar to that of hepatic cytochrome P-450 (5,12). It should be borne in mind that the quantitative immunohistochemical techniques used in this study reveal only the presence of the enzyme and not its activity. It is very likely, however, that the activity of NADPH–cytochrome *c* (P-450) reductase is greater in the centrilobular region than in the periportal region of the lobule, since it has recently been shown that centrilobular hepatocytes have far more NADPH available for monooxygenation reactions (13) and metabolize 7-ethoxycoumarin at a faster rate (6) than do those hepatocytes situated in the periportal region. It is, therefore, highly probable that centrilobular hepatocytes are more important in the Phase I metabolism of a majority of drugs and other foreign compounds than are periportal hepatocytes.

Acknowledgments

The authors wish to thank Professor E.D. Wills for his encouragement and helpful discussion and N.P. Jewell, M. Francis, and J. Schulman for their help with the statistical analyses.

Literature Cited

1. Baron J, Redick JA, Greenspan P, Taira Y: Immunohistochemical localization of NADPH-cytochrome c reductase in rat liver. Life Sci 22:1097, 1978
2. Baron J, Redick JA, Guengerich FP: An immunohistochemical study on the localizations and distributions of phenobarbital- and 3-methylcholanthrene-inducible cytochromes P-450 within livers of untreated rats. J Biol Chem 256:5931, 1981
3. Baron J, Redick JA, Guengerich FP: Effects of 3-methylcholanthrene, β-naphthoflavone, and phenobarbital on the 3-methylcholanthrene-inducible isozyme of cytochrome P-450 within centrilobular, midzonal, and periportal hepatocytes. J Biol Chem 257:953, 1982
4. Chayen J: Microdensitometry. In Biochemical Mechanisms of Liver Injury. Edited by TF Slater. Academic Press, London, 1978, p 257–291
5. Gooding PE, Chayen J, Sawyer B, Slater TF: Cytochrome P-450 distribution in rat liver and the effect of sodium phenobarbitone administration. Chem-Biol Interactions 20:299, 1978
6. Ji S, Lemasters JJ, Thurman RG: A fluorometric method to measure sublobular rates of mixed-function oxidation in the hemoglobin-free perfused rat liver. Mol Pharmacol 19:513, 1981
7. Kawabata TT, Guengerich FP, Baron J: An immunohistochemical study on the localization and distribution of epoxide hydrolase within livers of untreated rats. Mol Pharmacol 20:709, 1981
8. Lowry OH, Passonneau J: A Flexible System of Enzymatic Analysis. Academic Press, New York, 1972
9. Omura T, Takesue S: A new method for simultaneous purification of cytochrome b_5 and NADPH-cytochrome c reductase from rat liver microsomes. J Biochem (Tokyo) 67:249, 1970
10. Redick JA, Kawabata TT, Guengerich FP, Krieter PA, Shires TK, Baron J: Distributions of monooxygenase components and epoxide hydrolase within the livers of untreated male rats. Life Sci 27:2465, 1980

11. Seligman AM, Karnovsky MJ, Wasserkrug HL, Hanker JS: Nondroplet ultrastructural demonstration of cytochrome oxidase activity with a polymerizing osmophilic reagent, diaminobenzidine (DAB). J Cell Biol 38:1, 1968

12. Smith MT, Wills ED: Effects of dietary lipid and phenobarbitone on the distribution and concentration of cytochrome P-450 in the liver studied by quantitative cytochemistry. FEBS Lett 127:33, 1981

13. Smith MT, Wills ED: The effect of dietary lipid and phenobarbitone on the production and utilization of NADPH in the liver. Biochem J 200:691, 1981

14. Smith MT, Wills ED, Drew K, Maxwell C, Daly JR, Reader SCJ, Robertson WR: The use of an inexpensive, general purpose microcomputer in quantitative cytochemistry. Histochemistry 68:321, 1980

15. Sternberger LA, Hardy PH, Cuculis JJ, Meyer HG: The unlabeled antibody enzyme method of immunohistochemistry. Preparation and properties of soluble antigen-antibody complex (horseradish peroxidase-antihorseradish peroxidase) and its use in the identification of spirochetes. J Histochem Cytochem 18:315, 1970

16. Taira Y, Greenspan P, Kapke GF, Redick JA, Baron J: Effects of phenobarbital, pregnenolone-16α-carbonitrile, and 3-methylcholanthrene pretreatments on the distribution of NADPH-cytochrome c (P-450) reductase within the liver lobule. Mol Pharmacol 18:304, 1980

17. Taira Y, Redick JA, Baron J: An immunohistochemical study on the localization and distribution of NADPH-cytochrome c (P-450) reductase in rat liver. Mol Pharmacol 17:374, 1980

18. Taira Y, Redick JA, Greenspan P, Baron J: Immunohistochemical studies on electron transport proteins associated with cytochromes P-450 in steroidogenic tissues. II. Microsomal NADPH-cytochrome c reductase in the rat adrenal. Biochim Biophys Acta 583:148, 1979

19. Van Orden LS III: Quantitative histochemistry of biogenic amines. A simple microspectrofluorometer. Biochem Pharmacol 19:1105, 1970