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Effects of Toxic Agents at the Protein Level: Quantitative Measurement of 213 Mouse Liver Proteins following Xenobiotic Treatment[1]

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Effects of Toxic Agents at the Protein Level: Quantitative Measurement of 213 Mouse Liver Proteins following Xenobiotic Treatment. ANDERSON, N. L., GIERE, F. A., NANCE, S. L., GEMMELL, M. A., TOLLAKSEN, S. L., AND ANDERSON, N. G. (1987). *Fundam. Appl. Toxicol.* **8**, 39–50. By analyzing two-dimensional electrophoretic patterns of mouse liver proteins with a computerized image analysis system, we have observed quantitative changes in the abundance of more than 70 proteins in mice treated with various agents. Aroclor 1254, a mixture of polychlorinated biphenyls known to induce a broad spectrum of microsomal activity, induces the largest group of changes (60 proteins altered at $p < 0.001$ significance). Phenobarbital produces a small set of characteristic changes that forms part of the much larger Aroclor 1254 effect. Ibuprofen treatment produces a phenobarbital-like pattern of change, with the addition of at least one protein change not observed with any of the other treatments. Cycloheximide and carbon tetrachloride each induces a different characteristic pattern of protein alteration. We have assigned most of the mouse liver proteins to a specific subcellular fraction, and it appears that the predominant class of proteins altered by each compound is present in the soluble phase, rather than in the microsomal fraction. The ability to survey large numbers of tissue proteins for involvement in pharmacologic and toxic effects may allow a more comprehensive understanding of the mechanisms of action *in vivo* and provide new markers of tissue damage.

Proteins carry the principal responsibility for moment-to-moment operation of a living cell. The reactions of cellular metabolism are regulated by protein enzymes, and many of the architectural features of the cell depend on structural proteins. It is therefore natural to look to effects on proteins as important initial features of drug action, and as secondary consequences along the path to cell alteration or death.

Two-dimensional (2-D) electrophoretic protein mapping is a technique that allows the separation and visualization of up to several thousand proteins in a single analysis (O'Farrell, 1975; Anderson and Anderson, 1978a,b). This technology therefore has the potential to yield a much more comprehensive picture of protein effects than has been available previously. Through computer analysis of 2-D patterns (Anderson *et al.*, 1981), it is possible to quantitate the abundance of proteins quite accurately. More than 100 polypeptides can be measured on Coomassie blue-stained 2-D patterns of mouse liver proteins with coefficients of variation less than 15% (i.e., competitive with many enzymatic or immunochemical-specific protein tests) (Anderson *et al.*, 1985).

In a previous paper, we showed that Aroclor 1254, a mixture of polychlorinated bi-

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phenyls known to induce a range of microsomal enzymes (Alvares *et al.*, 1973), produced a variety of dose-related changes in the protein pattern of unfractionated mouse liver (Anderson *et al.*, 1986). On the basis of this preliminary experiment, it was suggested that analysis of liver proteins of treated animals could provide much more information per animal than could many current test protocols. Drug-induced changes in the synthesis or degradation of individual proteins should be visible, and information on the nature of such proteins should contribute to an understanding of drug action. A number of stringent criteria must be met, however, before this possibility can be realized. First, it must be shown that the effects of compounds having different mechanisms of action are indeed different. Unless some specificity is observed, the information obtained by measuring hundreds of proteins would be minimal. Second, it must be shown that there is some value in documenting effects on proteins of currently unknown function. Since the vast majority of proteins observed will be unidentified (at least as to enzyme or other recognizable name), the important question is whether other sorts of information, short of precise identification, can make observed effects valuable.

In this paper, we make an initial attempt at addressing both issues. In the first case, we use protein mapping with computerized data reduction to compare the patterns of liver proteins from replicate mice treated with high doses of five different compounds: Aroclor 1254, phenobarbital (known to induce a more restricted range of microsomal activity), ibuprofen (an analgesic and antiinflammatory compound used very widely in humans), cycloheximide (an inhibitor of eukaryotic protein synthesis), and carbon tetrachloride (a classic hepatotoxic agent). High doses are used, capable of leading to the deaths of some animals, to provide the best opportunity to observe characteristic effects. As to the second issue, we use cell fractionation techniques to divide the proteins observed into categories associated with the major cell organelles: the nucleus, mitochondria and lysosomes, microsomes (endoplasmic reticulum and Golgi apparatus), and soluble phase. This approach provides a basic piece of information about each protein in the pattern, despite the lack of a formal identification for all but a few. In particular, this fledgling protein database allows us to ask whether the major effects of any of the test compounds are restricted to the microsomal cell fraction, the site of most known liver detoxification apparatus.

METHODS

Male C57BL/6JANL mice between 4 and 5 months of age were given xenobiotic or sham treatments by stomach tube. Each group consisted of six mice assigned by randomizing individuals from five cages. Hydrophobic agents were administered in peanut oil; treated mice received 0.2 ml of 25% carbon tetrachloride (2.5 ml/kg), 8 mg/ml ibuprofen (80 mg/kg), or 25 mg/ml Aroclor 1254 (250 mg/kg). Control mice received 0.2 ml of peanut oil alone. Phenobarbital was administered in food pellets (0.1% by weight; approximately 200 mg/kg ingested). Cycloheximide was administered by gavage as 1 ml of 0.8 mg/ml in water (40 mg/kg). All treatments were given daily for 7 days, except for cycloheximide, which was administered for only 4 days due to high toxicity. Several mice did not survive treatment at these doses: two mice receiving carbon tetrachloride, two receiving ibuprofen, and one receiving cycloheximide died before completion of the treatment period and were not analyzed. Surviving mice were killed by cervical dislocation on the day following the last treatment, and the apical end of the left lobe of the liver was homogenized in a solution of 9 M urea, 2% NP-40 detergent, 2% mercaptoethanol, and 2% ampholytes (pH 9–11), at a final pH of 9.5. Solubilized samples were centrifuged at 100,000*g* for 30 min, and the supernatants were stored at −80°C.

Liver freshly excised from male C57BL/6JANL mice was used as starting material for several classical fractionation protocols. Mitochondria (Chappall and Hansford, 1972), microsomes (Tata, 1972), and nuclei (Blobel and Potter, 1966) were prepared according to the methods referenced. The soluble phase was prepared by homogenizing 1 g of liver (chopped with scissors and washed with 0.25 M sucrose) in 4 ml 0.25 M sucrose with a Dounce homogenizer. This homogenate was centrifuged for 1 hr at 105,000*g*, 5°C, and the supernatant was collected. All fractions were prepared for 2-D electrophoresis by the addition of 9 M urea, 2% NP-40, 2% mercaptoethanol, and 2% ampholytes (LKB, pH 9–11).

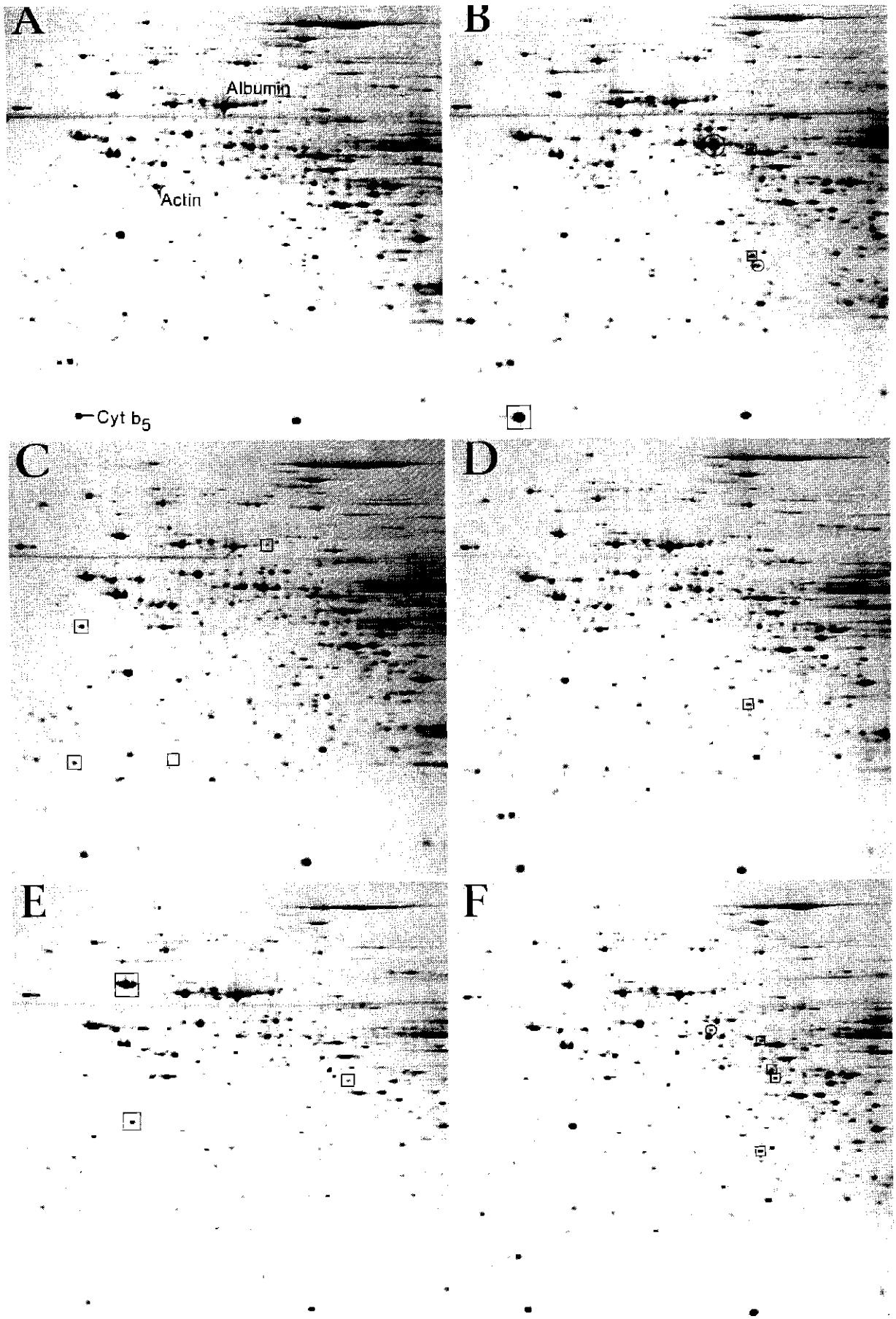

FIG. 1. Two-dimensional protein patterns of livers obtained from individual mice, either untreated (A) or treated with Aroclor 1254 (B), cycloheximide (C), phenobarbital (D), carbon tetrachloride (E), or ibuprofen (F). Positions of serum albumin, cellular non-muscle actin, and cytochrome b_5 are indicated in (A) for reference. Protein abundance changes that are evident by simple visual inspection are enclosed in symbols: circles enclose proteins in which a change occurs apparently specific to one treatment, while squares enclose changes common to two or more treatments.

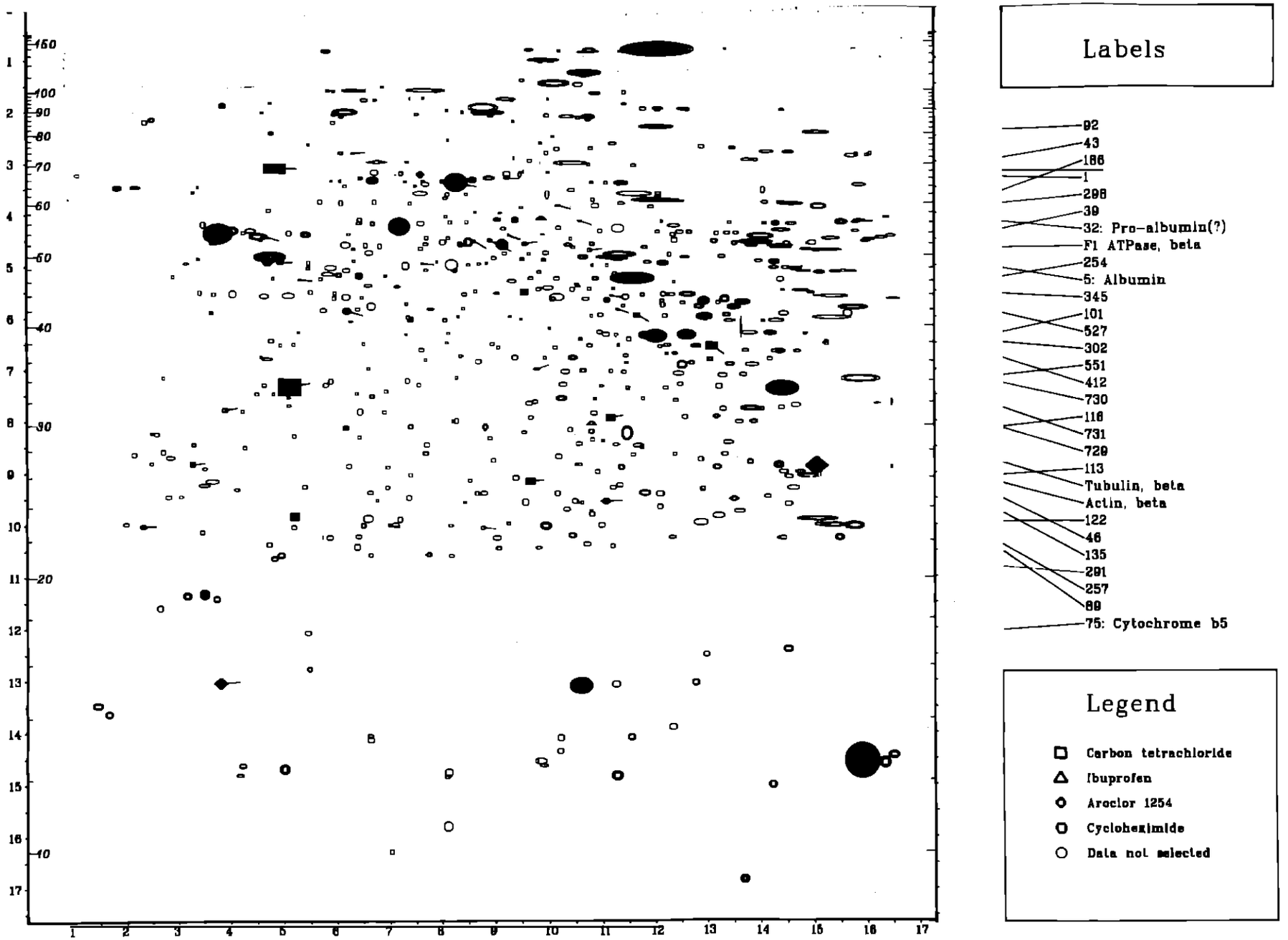

FIG. 2. Schematic map of the mouse liver protein pattern showing locations of individual proteins affected by drug treatment. The vertical scale inside the plot border shows approximate SDS molecular mass as derived from a series of 18 plasma protein and other markers (Anderson *et al.*, 1984b), while the exterior scales indicate length (in centimeters) on the original master gel. Proteins labeled on the right can be located by following the line emanating from the label to its end as a short line segment extending from the spot. As indicated in the lower right corner, proteins changing ($p < 0.001$) with various agents are plotted as symbols, while other proteins are plotted as elipses. The number of contours (size and density of the spot) are directly related to the abundance of the protein in untreated liver.

TABLE I

MEAN ABUNDANCES (AND STANDARD DEVIATIONS) FOR SELECTED SPOTS INDICATED IN FIG. 2[a]

MSN	Control	CCl_4	Ibuprofen	Phenobarbital	Aroclor 1254	Cycloheximide
1	4,282 (507)	13,446 (1186)*	4,324 (530)	4,682 (147)	3,938 (319)	5,401 (626)
9	2,429 (223)	3,810 (409)	2,777 (286)	2,502 (195)	3,246 (220)*	2,076 (134)
32	766 (214)	2,164 (671)	869 (173)	1,181 (260)	939 (216)	211 (185)
39	5,915 (319)	1,584 (267)*	4,715 (595)	3,814 (1070)	2,180 (314)*	5,917 (481)
43	1,413 (127)	666 (117)	1,221 (313)	1,305 (284)	9,916 (2775)*	952 (153)
45	933 (134)	983 (65)	1,673 (69)*	1,051 (55)	1,478 (97)*	809 (263)
46	1,047 (85)	439 (83)	580 (107)	786 (80)	420 (48)*	1,177 (154)
51	1,100 (52)	743 (104)	872 (64)	931 (58)	753 (56)*	1,139 (34)
62	1,266 (252)	107 (38)*	1,149 (39)	830 (255)	589 (125)*	493 (223)
67	1,210 (265)	802 (75)	1,138 (228)	1,090 (178)	609 (93)*	1,264 (294)
75	2,675 (822)	2,350 (357)	3,533 (396)	4,645 (237)	11,295 (232)*	3,325 (155)
89	2,683 (244)	1,613 (64)*	1,957 (86)	1,774 (177)	1,931 (152)*	3,254 (85)
91	1,095 (94)	442 (25)	994 (168)	906 (115)	758 (99)*	1,135 (93)
92	349 (23)	228 (20)	245 (24)	280 (27)	105 (27)*	290 (21)
101	1,783 (125)	430 (24)*	1,130 (111)	1,075 (135)	417 (59)*	1,571 (52)
116	1,093 (106)	300 (43)*	808 (198)	988 (219)	233 (22)*	1,407 (266)
122	848 (66)	990 (110)	1,380 (125)	1,252 (142)	2,643 (197)*	817 (116)
135	829 (61)	278 (59)*	616 (65)	586 (39)	372 (38)*	741 (55)
166	476 (37)	183 (31)*	317 (52)	410 (78)	348 (35)*	398 (15)
182	354 (28)	291 (63)	334 (19)	354 (134)	289 (20)	161 (31)*
183	624 (68)	111 (34)*	318 (60)	592 (119)	446 (79)	465 (58)
186	531 (65)	298 (28)	622 (70)	1,169 (299)	1,522 (234)*	502 (58)
223	406 (65)	619 (36)	1,104 (15)*	514 (240)	935 (162)*	407 (42)
254	320 (55)	929 (84)*	335 (21)	380 (41)	277 (34)	449 (17)
284	275 (44)	521 (71)	337 (30)	378 (55)	394 (45)	652 (86)*
291	356 (35)	393 (72)	557 (47)	667 (130)	906 (54)*	430 (32)
302	196 (15)	298 (33)	242 (14)	261 (5)*	355 (14)*	207 (9)
345	170 (17)	266 (27)	148 (24)	179 (35)	347 (22)*	225 (20)
412	97 (13)	306 (50)	120 (18)	133 (20)	187 (23)*	87 (10)
424	187 (23)	231 (31)	237 (39)	216 (33)	274 (22)*	276 (13)*
483	94 (24)	187 (4)*	105 (27)	156 (109)	164 (70)	108 (7)
527	65 (29)	152 (26)	243 (21)*	116 (28)	208 (35)*	57 (7)
551	73 (29)	358 (18)*	133 (23)	141 (24)	289 (32)*	101 (52)
729	U	546 (98)	438 (127)	29 (—)	170 (39)	U
730	U	592 (101)	978 (117)	U	873 (78)	U
731	170 (136)	66 (34)	294 (38)	113 (46)	226 (—)	U

[a] All values are in units of pixel-grey levels divided by 10. MSN stands for master spot number, the serial number assigned to each protein spot in this experiment. An asterisk (*) indicates values different from the control group at significance $p < 0.001$. "U" indicates a situation where the spot was not detected on any gel of the group.

Two-dimensional electrophoresis was performed with the 7 × 7-in. ISO–DALT system (Anderson and Anderson, 1978a,b) in batches of 20 gels. First-dimension gels contained a 1:1 mixture of Biolyte pH 3–10 and pH 5–7 ampholytes (Bio-Rad Laboratories), and second-dimension 9–17% linear polyacrylamide gradient slab gels were prepared by a computer-controlled casting device. Gels all were stained with Coomassie brilliant blue and were treated as described (Anderson *et al.*, 1985). Each of the six control samples was analyzed twice (on different batches of gels), as were several of the experimental samples.

The TYCHO 2-D gel data processing system (Anderson *et al.*, 1981) was used to analyze 2048 × 2048 pixel gel images acquired with a specially modified Eikonix 785 scanner. Gels were matched together and stretched into registration (Taylor *et al.*, 1983). Each gel was scaled (to eliminate any differences in total protein applied to

TABLE 2

DISTRIBUTION OF PROTEIN ABUNDANCE CHANGES SIGNIFICANT AT $p < 0.01$ AND $p < 0.001$ IN FIVE TREATMENT GROUPS, AND OVERLAP OF OBSERVED CHANGES BETWEEN GROUPS[a]

		CCl_4	Ibuprofen	Phenobarbital	Aroclor 1254	Cycloheximide
CCl_4	$p < 0.01$	67	12/12	7/7	39/42	13/14
	$p < 0.001$	13	0	0	9/9	0
Ibuprofen	$p < 0.01$		27	3/3	22/22	6/7
	$p < 0.001$		3	0	3/3	0
Phenobarbital	$p < 0.01$			11	8/11	2/3
	$p < 0.001$			1	1/1	0
Aroclor 1254	$p < 0.01$				96	14/19
	$p < 0.001$				60	1/1
Cycloheximide	$p < 0.01$					27
	$p < 0.001$					3

[a] Figures show the number of proteins that were observed to change in abundance due to xenobiotic treatment with $p < 0.01$ or $p < 0.001$ as determined by a conventional statistical t test interpreted with a very conservative value for number of degrees of freedom. The number of protein changes in common between each pair of compounds is shown as the number changed in the same sense (i.e., both compounds increase the protein, or both decrease it) over the total number of commonalities.

the gel) by setting the total of all matched spots of abundance less than 30,000 units equal to the sum of the corresponding spots on the master gel. Since this scaling was typically done over 450 spots for each gel, it provides a good method for normalizing individuals. To select a set of reproducible and well-behaved protein spots, we selected those proteins that were matched properly on all 12 control gels. These 213 proteins form the data set analyzed here. A statistical t test (Sokal and Rohlf, 1981) was used to evaluate the significance of quantitative differences from control values. Because the variance of protein abundance measurements is not independent of abundance, we used a value for the number of degrees of freedom equal to the minimum value for control or treated groups. This assumption yields more conservative estimates of significance than do some other common approaches. Principal component analysis was performed with the ARTHUR software package (Infometrix, Inc.) essentially as described (Anderson *et al.*, 1984a).

RESULTS

Two-dimensional protein maps of unfractionated mouse liver typically show 600–800 protein spots when stained with Coomassie brilliant blue. Figure 1 shows the appearance of representative control and treated samples prepared from the mice in the present experiment. All the compounds included here produce reproducible protein changes that can be detected by eye. Figure 2 shows the major changes presented schematically on a master protein pattern and provides molecular weight and spot identification information. Table 1 presents protein abundance data on selected spots for the control and treatment groups. A statistical analysis of quantitative measurements derived from these patterns turns up additional differences and provides a measure of the statistical significance of individual effects. The numbers of proteins found to show quantitative changes at t-test significances of $p < 0.01$ and $p < 0.001$ are shown in Table 2. In addition, Table 2 shows the overlap of these sets between compounds. Each compound produces some effects in common with Aroclor 1254, and in almost all cases these changes are in the same sense (increasing or decreasing abundance). While differences at a $p < 0.01$ significance are generally acceptable as reliable effects (in this case almost all are verifiable by eye) it is important to realize that among 213 variables two or three should show differences at this

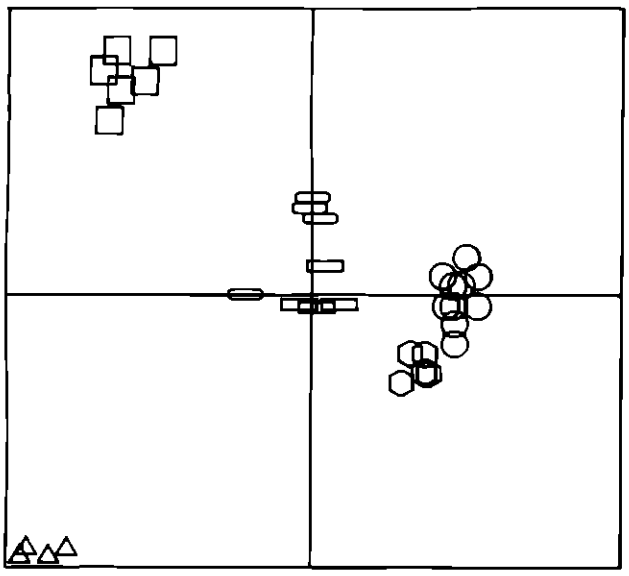

FIG. 3. Principal component analysis of the patterns of change caused by treatment with various xenobiotics. The horizontal dimension corresponds to the first principal component (representing 84.6% of total data variance), and the vertical dimension to the second principal component (7.7% of data variance). Each group of animals forms a recognizable cluster: controls (circles), carbon tetrachloride (triangles), ibuprofen (ovals), phenobarbital (rectangles), Aroclor 1254 (squares), and cycloheximide (hexagons). The greatest intragroup heterogeneity appears in the ibuprofen group, where one individual seems substantially different from the others. The cycloheximide-treated group is better resolved from the control and other groups by the third principal component (not shown).

level by chance. Since, however, each of the compounds tested shows 11 or more effects at $p < 0.01$, and since each of the agents shows some effect at the more rigorous level of $p < 0.001$, we can conclude that statistically reliable effects are observed with each agent.

Mixed chlorinated hydrocarbons produce by far the most widespread pattern of change observed in this experiment (96 proteins significantly changed in abundance at $p < 0.01$, and 60 at $p < 0.001$).

At the dose delivered in this experiment, phenobarbital produces a small number of effects, most of which are also observed with Aroclor 1254. Only one is significant at $p < 0.001$.

Ibuprofen also induces a small set of changes, and most of these are once again observed with Aroclor 1254. However, one protein detected by visual analysis (circle in Fig. 1F; spot No. 731 in Fig. 2) shows an increase apparently unique to this compound out of the group studied. Its molecular mass is approximately 54,000 Da (Fig. 2). Since the protein is not reliably detected in untreated liver, it was not included in the set of 213 proteins initially chosen for study and its primary subcellular location was not revealed by subcellular fractionation experiments. We have called this protein Ibu:1, in line with previous protein-mapping nomenclature (Anderson *et al.*, 1981).

Carbon tetrachloride produces large abundance changes in a variety of cellular proteins, many of which are different from the effects of Aroclor 1254. This divergence

TABLE 3

LOCATION AMONG CELL FRACTIONS OF THE PROTEINS AFFECTED ($p < 0.001$) BY VARIOUS XENOBIOTICS[a]

	Soluble	Mitochondria	Microsomes	Nuclei	Unassigned
CCl_4	8	2	1	0	2
Ibuprofen	1	1	0	0	1
Phenobarbital	1	0	0	0	0
Aroclor 1254	32	11	6	0	11
Cycloheximide	2	0	0	0	1

[a] Each protein found to change at $p < 0.001$ was located in the cell fractions, and was assigned to one fraction only if there was a clear predominance over all others. In cases where two or more fractions showed appreciable amounts of a protein, the location is assumed unknown.

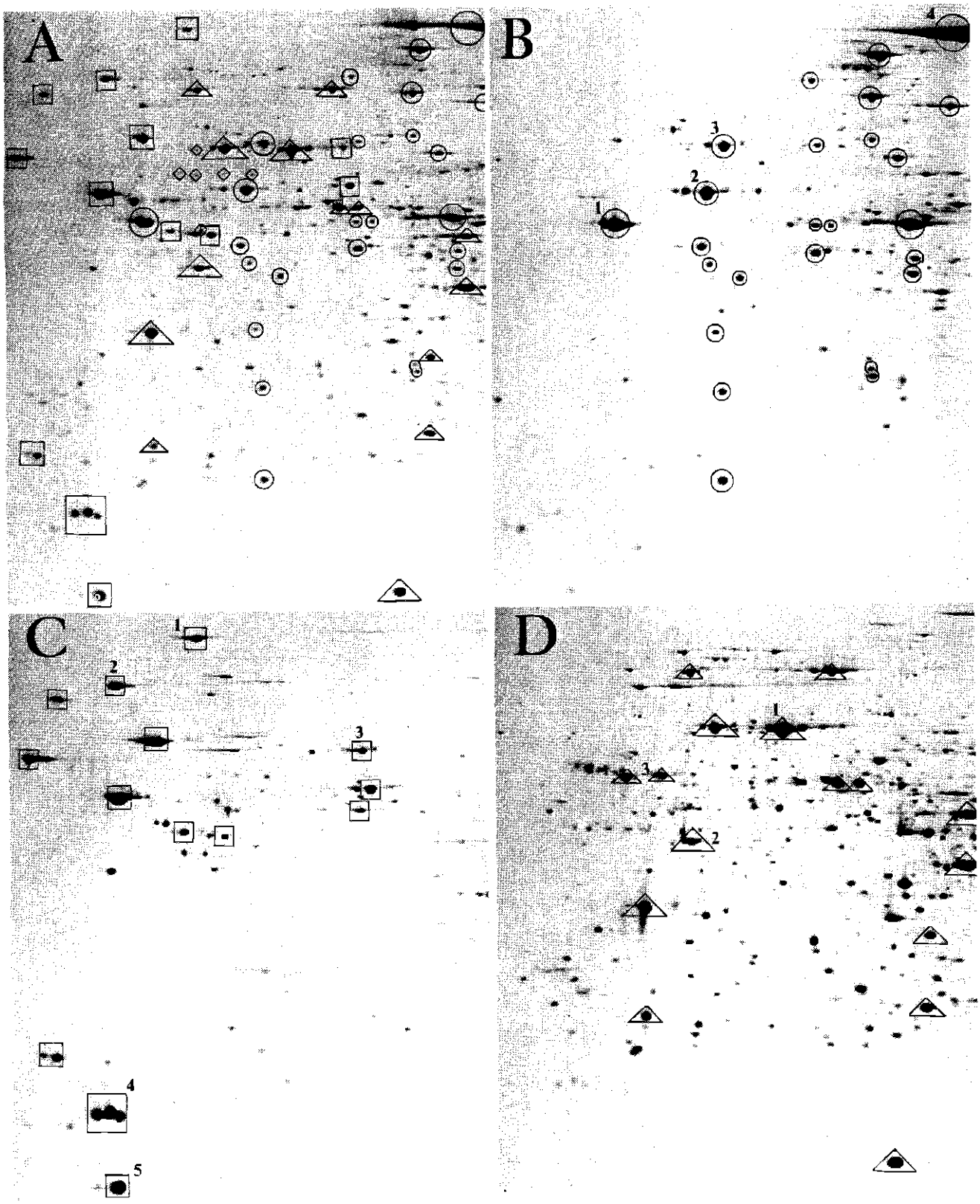

FIG. 4. Two-dimensional protein patterns of unfractionated liver (A), a mitochondrial fraction (B), a microsomal fraction (C), and the soluble phase (D). Major proteins of each fraction are indicated in the unfractionated pattern in (A) and in the respective fraction pattern: mitochondrial proteins are enclosed in circles, microsomal proteins in squares, and some soluble proteins in triangles. Nuclear proteins, which represent only minor components in this pH range, are enclosed in diamonds. Circles labeled 1–3 are the proteins designated Mitcon:1–3 in previous studies of human cells (Anderson, 1981). Mitcon:1 is the β subunit of the F1 ATPase, and is hence part of the inner membrane. Mitcon:2 and :3 are mitochondrial matrix polypeptides (Anderson, 1985). Circle 4 is likely to be carbamyl phosphate synthetase by analogy with the rat mitochondrial protein pattern (Henslee and Srere, 1979). Squares 1 and 2 are known by

might be expected, since lipid peroxidation and associated reactions are thought to be responsible for the toxicity of carbon tetrachloride. One major microsomal polypeptide (spot No. 1, topmost square in Fig. 1E) is dramatically increased in abundance while others, including a range of soluble-phase proteins, are decreased.

Cycloheximide produces a small number of changes of a characteristic nature. A microsomal spot, identified tentatively as the proalbumin polypeptide on the basis of charge and molecular mass (see below), is decreased dramatically, as would be expected to follow decreased synthesis of this major liver product and its subsequent clearance from the endoplasmic reticulum and Golgi apparatus. At least one protein is increased following cycloheximide treatment, suggesting that decreased protein synthesis may lead to decreased turnover of some molecules. Finally, one microsomal glycoprotein present in the Golgi (see below) appears to become at least partially unglycosylated. The rather extended spot corresponding to the protein becomes two spots, one very slightly below the normal location, just distinguishable in overlap comparisons of the patterns. This change is one of the major effects when cultured cells are treated with tunicamycin, an inhibitor or asparagine-linked glycosylation (NLA, unpublished), and suggests that cycloheximide treatment interferes, directly or indirectly, with protein glycosylation in the Golgi.

The relationship between the different drug-induced patterns of change was examined by the use of a multivariate statistical approach. Figure 3 shows the positions of all individual patterns analyzed with respect to the first two largest principal components of a conventional principal component (PC) analysis (Anderson *et al.*, 1984a). Each treatment produced a relatively tight distribution of individuals, separate from the other treatments. The largest discrepancy occurs with ibuprofen, where one individual differs significantly from the other three. All treatments show some excursion to the same side of the controls in the first component. This component may thus represent a pattern of protein change associated with liver toxicity generally, while the second (and possibly further) component represents more specific effects.

Cell fractions prepared through the classical methods of differential centrifugation were analyzed to assign individual proteins to cell compartments (Table 3). As shown in Fig. 4, the three major fractions (soluble, mitochondrial, and microsomal) account for a large majority of the proteins. The nuclear fraction contains only a few minor specific proteins detectable in the pH range investigated here (diamonds in Fig. 4A); the principal nuclear proteins (histones, etc.) are too basic to focus on these gels and are visible instead on BASO gels (Willard *et al.*, 1979).

The mitochondrial proteins detected are generally neutral or basic and most have polypeptide molecular masses greater than 40,000 Da. Three major mitochondrial proteins (labeled 1–3 in Fig. 4A) have previously been characterized on 2-D gels as Mitcon:1, :2, and :3. Mitcon:1 is the β subunit of the F1 ATPase and is highly conserved in position among the mammals. Mitcon:2 and :3 are mitochondrial matrix polypeptides (Anderson, 1985) of unknown function.

The microsomal proteins resolved on these gels are, by contrast, primarily acidic molecules. The two of highest molecular mass (boxes 1 and 2 in Fig. 4C) are known from

analogy with studies of human proteins to be glycoproteins not present on the cell surface (and hence likely to reside in internal membranes). Square 2 is likely to be a major Golgi apparatus protein (Lin *et al.*, 1982). Square 3 is likely to be the proalbumin polypeptide, and square 5 is almost certainly cytochrome b_5. Square 4 is a protein expressed predominantly in males though at quite variable abundance. Triangle 1 is serum albumin, triangle 2 is actin, and triangles 3 are β and α tubulin (left to right).

studies in human cells to be highly glycosylated but not present on the cell surface. The second highest (box 2) is known to be associated with the Golgi apparatus (Lin *et al.*, 1982). Several low molecular mass microsomal proteins are resolved, the smallest of which (box 5) is likely to be cytochrome b_5, on the basis of similarity to the purified rat protein (Anderson *et al.*, 1986).

One microsome-associated spot (box 3 in Fig. 4) occupies a position expected for the proalbumin polypeptide: the known sequence of the rat albumin primary translation product (presumably similar to the mouse sequence) indicates that the *n*-terminal peptide extension (removed after the signal peptide) has a net charge of +3, placing this molecule three charge shift units to the right (more basic) and very slightly above the position of albumin, precisely where the candidate is observed. Proalbumin should be located primarily in the endoplasmic reticulum and perhaps the Golgi apparatus and should therefore appear mainly in the microsomal cell fraction as observed. The reduction in abundance of this protein following cycloheximide treatment is consistent with its role as a short-lived intermediate form of the plasma protein.

DISCUSSION

The results presented here clearly demonstrate effects of high-dose xenobiotic treatment on the protein composition of livers of treated mice. Two-dimensional protein mapping allowed us to measure the abundance of more than 200 proteins in each mouse and thereby uncover a variety of specific changes due to each treatment. Although very few of the proteins measured have been identified so far, a cell fractionation approach provided sufficient characterization of most proteins to shed some light on the nature of the effects observed. Somewhat surprisingly, the microsomal proteins did not account for a majority of changes induced by any of the compounds. Even Aroclor 1254 (which causes substantial proliferation of the smooth endoplasmic reticulum and induces a range of cytochrome *P*-450s) produced changes concentrated elsewhere in the cell. Although cytochrome b_5 (primarily a microsomal protein) is clearly induced, most affected proteins are either cytosolic or mitochondrial. Such results suggest that high-dose effects of these compounds may involve several subcellular sites.

In particular, this result indicates that many changes in cellular metabolism take place in the cytoplasm of cells treated with agents best known for their effects on microsomal enzymes. Vlasuk and co-workers (1980, 1982), in a series of elegant studies of microsomal enzyme inducers in the rat, used two-dimensional electrophoresis to examine exclusively the microsomal proteins. Their system was optimized for analysis of cytochrome *P*-450s (and was highly successful in this regard), yet because of the prefractionation to enrich microsomes, effects on the soluble and mitochondrial proteins were not observed. The system used here is more comprehensive, since unfractionated liver is analyzed, but still not complete due to the absence of very basic proteins from these maps. Cytochrome *P*-450, for example, appears to be too basic to appear on gels of the type used here (Anderson *et al.*, 1986), and would require use of BASO gels in our system (Willard *et al.*, 1979). Nevertheless, we believe that the present system allows examination of a much broader spectrum of proteins than do previous studies, and that through the use of computerized quantitation it allows much more subtle effects to be observed.

One of the principal reasons for examining many individual proteins is the possibility of discovering a specific protein involved in a particular pharmacologic effect. Protein Ibu:1 was found to be increased substantially by ibuprofen treatment, while most other proteins altered by ibuprofen are similarly changed by Aroclor 1254 and may represent part of a general liver response. Ibu:1 thus represents an example of a candidate specific

indicator protein. A general survey of other mouse organs would reveal whether the protein is increased at the sites of major overdose toxicity; if so, Ibu:1 could merit further investigation as a specific indicator of ibuprofen intoxication and as a clue in the search for the mechanism of toxicity. If such an effort were desirable, a range of biochemical techniques exists for translating an observation from a 2-D gel to the realms of routine testing and molecular biology. By preparing a series of 2-D separations from treated animals, sufficient Ibu:1 protein can be obtained to allow microsequence analysis and subsequent identification of the associated gene, as well as preparation of a specific antibody. Immunohistochemical methods, in concert with cell fractionation, can then pinpoint the subcellular location of the protein. Given such information, a range of possible functions can be inferred and tested. Thus, it may be possible to proceed from a protein-mapping survey to the detailed study of one or more molecules likely to be involved somehow in the drug effect.

A potential drawback of the protein-mapping approach is that it does not measure the "effectiveness" of the proteins observed (enzymatic activity, etc.), but only the amount of polypeptide present. Thus, if a drug inhibits an enzyme by noncovalent binding and produces no change in the amount of enzyme, then a major metabolic effect could occur without a change in the protein pattern. Alternatively, a drug could induce or eliminate a protein, but one of such low abundance that it is not detected on tissue protein maps. Negative results are thus likely to be obtained for at least some compounds. A major feature of the data presented here is that they demonstrate experimentally that many compounds do produce observable effects. Whether these are due to gene regulation events triggered by the xenobiotic (induction by Aroclor 1254 or phenobarbital, or translation shutoff by cycloheximide) or to the selective destruction of cell types or organelles (as may occur following carbon tetrachloride treatment), the result ought to be interpretable against a sufficient database of information. The construction of such a comprehensive database for mouse, and ultimately rat, liver is likely to yield fundamental new insights in toxicology and pharmacology.

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