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# Subcellular Localization of Membrane-Bound Aryl-Hydrocarbon Hydroxylase and NAD(P)H-Dependent Reductase Activities in Mouse Liver

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The subcellular distribution of aryl-hydrocarbon hydroxylase, NADPH-cytochrome *c* reductase, NADH : cytochrome *c* reductase, and NADH : cytochrome $b_5$ reductase activities in mouse liver was studied using the biochemical membrane markers microsomal glucose-6-phosphatase, mitochondrial cytochrome *c* oxidase, and plasma membrane 5′-nucleotidase. The rate of appearance of activity of 3-methylcholanthrene-induced aryl-hydrocarbon hydroxylase in the microsomes of C57BL/6N mice is more than twice as rapid as that in the nuclear envelope. The nuclear fraction contains less than 1% of the total cellular activities of the hydroxylase and all three reductases. All detectable basal activity of aryl-hydrocarbon hydroxylase in the nuclear fraction of control C57BL/6N and DBA/2N and 3-methylcholanthrene-treated DBA/2N mice and all detectable activities of NADPH:cytochrome *c*, NADH:cytochrome *c*, and NADH:catochrome $b_5$ reductase in the nuclear fraction of control and 3-methylcholanthrene-treated C57BL/6N and DBA/2N mice can be completely accounted for by the degree of microsomal fragment contamination (as assessed by the microsomal marker glucose-6-phosphatase). These data raise doubts about certain previous reports of 'nuclear' enzyme activities in which microsomal contamination was not taken into account. However, there is more induced activity of aryl-hydrocarbon hydroxylase in the nuclear fraction of 3-methylcholanthrene-treated C57BL/6N mice than can be accounted for by the degree of microsomal membrane conrribution.

The murine *Ah* locus is known to regulate the induction by certain polycyclic aromatic chemicals of numerous drug-metabolizing enzyme activities such as aryl-hydrocarbon hydroxylase associated with cytochrome $P_1$-450. The expression of 3-methylcholanthrene-inducible hydroxylase in nuclear membranes, like that in microsomal membranes, thus appears to be controlled by the *Ah* regulatory gene.

Kasper first suggested the presence of a monooxygenase system mediated by cytochrome *P*-450 in liver nuclear membranes [1]. Since then, there have appeared numerous conflicting reports. Based upon data from induction ratios, sensitivity to inhibitors, metabolite profiles, and/or immunological techniques, various laboratories have concluded that the nuclear and microsomal membrane systems are either distinctly different [1–5] or the same [6–13]. Specific activity of nuclear aryl-hydrocarbon hydroxylase has been reported to represent 7% to 11% [1], 15% to 20% [14], or 2% to 12% [15] of the specific activity of microsomal aryl-hydrocarbon hydroxylase in rat liver. Studies comparing microsomal and 'nuclear' fractions have included the metabolism of benzo(*a*)-pyrene [4, 7, 14, 16–25], aflatoxin $B_1$ [9, 13], 2-acetylaminofluorene [26–28], 3-methylcholanthrene [29], carbon tetrachloride and chloroform [30], and styrene [31].

There have been numerous conflicting publications concerning the subcellular localization of the membrane-bound monooxygenase system mediated by cytochrome *P*-450 (reviewed in [14, 16]). Important questions that remain unanswered include: (a) whether the nuclear and microsomal membrane systems are different or the same; (b) how much the nuclear membrane drug-metabolizing system contributes to

*Abbreviations.* B6, the C57BL/6N inbred mouse strain; D2, the DBA/2N inbred mouse strain; buffer A, 50 mM Tris-Cl pH 7.5, 25 mM KCl, and 5 mM $MgCl_2$.

*Enzymes.* Aryl-hydrocarbon [benzo(*a*)pyrene] hydroxylase (EC 1.14.14.1); NADPH:cytochrome *c* reductase or NADPH:cytochrome *c* oxidoreductase (EC 1.6.2.4); NADH:cytochrome *c* oxidoreductase or NADH:cytochrome *c* reductase (EC 1.6.99.3); NADH:cytochrome $b_5$ reductase or NADH:cytochrome $b_5$ oxidoreductase (EC 1.6.2.2); glucose-6-phosphatase (EC 3.1.3.9); cytochrome *c* oxidase (EC 1.9.3.1); and 5′-nucleotidase (EC 3.1.3.5).

the overall metabolism of carcinogens; and (c) the relative importance of the nuclear and microsomal systems in generating ultimate carcinogens (reactive intermediates) that bind covalently to intranuclear DNA. Basal and inducible activities of aryl-hydrocarbon hydroxylase have usually been studied as a marker for the monooxygenase system mediated by cytochrome *P*-450, because of the sensitivity of the enzyme assay and the presumed importance of this enzyme in polycyclic hydrocarbon carcinogenesis.

To our knowledge, all previous studies on the subcellular localization of the monooxygenase system have relied on the apparent purity of nuclei and microsomes, as judged by microscopic examination. With highly sensitive electron paramagnetic resonance techniques, however, it is possible to detect mouse-liver microsomal contamination of mitochondrial fractions and mitochondrial contamination of microsomal fractions (even after six careful resuspensions and recentrifugations) although the fractions appear 'pure' by light and electron microscopy [32] (T. Kakefuda and D. W. Nebert, unpublished data). In this report we used membrane marker enzymes to assess biochemically the subcellular localization of activity of hepatic aryl-hydrocarbon hydroxylase in control and 3-methylcholanthrene-treated B6 and D2 inbred mice.

## MATERIALS AND METHODS

### *Chemicals*

3-Methylcholanthrene, benzo(*a*)pyrene, NADPH, NADH, glucose 6-phosphate, and cytochrome *c* were purchased from Sigma Chemical Co. (St Louis, MO). The source of all other chemicals is indicated in the references cited [33–40]. 3-Methylcholanthrene and benzo(*a*)pyrene were routinely recrystallized from warm benzene by the addition of cold methanol [35].

### *Animals*

B6 and D2 mice were obtained from the Veterinary Resources Branch (National Institutes of Health, Bethesda, MD). Sexually immature weanlings of either sex (4–6-weeks-old) were used. Mice were housed (up to six per cage) in plastic cages with hardwood bedding and were exposed to 14 h light/10 h dark each day and were permitted free access to water and food (Purina NIH Open Formula Rat Mouse Ration or Purina Laboratory Chow).

### *Treatment of Mice and Preparation of Subcellular Fractions*

3-Methylcholanthrene in corn oil was administered as a single intraperitoneal dose (200 mg/kg) and the mice were killed at indicated times during the next 48 h. Controls received intraperitoneally the corn oil alone (25 ml/kg).

After the mice were killed, all subcellular fraction preparative operations were carried out on crushed ice or at 4 °C. Liver from four mice were combined at every time-point and were homogenized with a teflon pestle tissue grinder (0.13–0.18 mm clearance) in five volumes of cold 0.25 M sucrose in buffer A. The homogenate was then passed through four layers of cheesecloth. The subcellular fractionation was carried out according to the classical method of De Duve et al. [33]. The pellet after centrifugation at $600 \times g$ for 10 min is the crude nuclear fraction (*vide infra*). Supernatant material from the $600 \times g$ centrifugation was then centrifuged at $3200 \times g$ for 10 min, and this pellet is designated the M fraction. The resultant supernatant material was centrifuged at $12000 \times g$ for 20 min, and this pellet is designated the L fraction. Lastly, the resultant supernatant material was centrifuged at $105000 \times g$ for 60 min to separate the microsomal pellet, or P, fraction from the postmicrosomal supernatant, or S, fraction. The M and L fractions were each washed twice with one-fourth as much volume of buffer A as had been used initially and were recentrifuged at the same designated speeds as described above; the supernatant material from these washes was added to the original supernatant material before the next higher centrifugation was performed.

The crude nuclear fraction was resuspended in 0.25 M sucrose in buffer A and purified by the method of Blobel and Potter [34], with the use of 2.3 M sucrose in buffer A as a discontinuous sucrose density gradient. This purified fraction is designated fraction N. All particulate fractions were resuspended in buffer A with 30% glycerol for assay.

Freezing and storage of the liver at −70 °C did not affect total enzymic activities but did result in significant and nonpredictable changes in distribution of activities among the various subcellular fractions. All data were therefore obtained on fresh, nonfrozen fractions and were repeated several times to ensure reproducibility.

### *Enzyme Assays*

Aryl hydrocarbon hydroxylase activity was measured with benzo(*a*)pyrene as substrate [35]. One unit of activity, U, is defined as that amount of enzyme catalyzing in 1 min at 37 °C the formation of hydroxylated products causing fluorescence equivalent to that of 1.0 pmol of the 3-hydroxybenzo(*a*)pyrene recrystallized standard.

NADPH-cytochrome *c* reductase and NADH-cytochrome *c* reductase were measured by determining the formation of reduced cytochrome *c*, having an

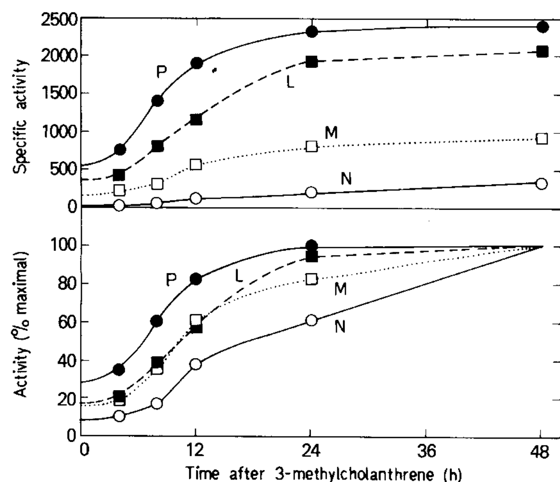


Fig. 1. *Aryl-hydrocarbon hydroxylase induction in B6 mouse liver as a function of time after a single dose of 3-methylcholanthrene had been given intraperitoneally*. The hydroxylase specific activities in the four subcellular particulate fractions are shown at top. Aryl-hydrocarbon hydroxylase activity as a percentage of the maximal attained is illustrated at bottom, with 48 h taken as the time at which 100% of the activity of each fraction had been reached. Preparation of the nuclear (N), crude mitochondrial (M), crude lysosomal (L), and crude microsomal (P) pellets are described under Materials and Methods

absorption coefficient at 550 nm of 18.5 $mM^{-1}$ $cm^{-1}$ [36]. NADH-cytochrome $b_5$ reductase was measured by following the production of reduced cytochrome $b_5$; the absorption coefficient for cytochrome $b_5$ ($\Delta A$ between 424 and 409 nm) is 163 $mM^{-1}$ $cm^{-1}$ [37]. Glucose-6-phosphatase and 5′-nucleotidase, marker enzymes of microsomal and plasma membranes respectively, were assayed by determining the formation of inorganic $PO_4$ [38]. Cytochrome *c* oxidase, a mitochondrial inner membrane marker enzyme, was assayed by the method of Cooperstein and Lazarow [39].

Except for aryl-hydrocarbon hydroxylase activity, one unit, U, of all the other enzyme activities is defined as that amount of enzyme catalyzing in 1 min the formation of 1.0 nmol of product (reduced cytochrome *c* or $b_5$ or inorganic phosphate or oxidized cytochrome *c*). Specific activity of all enzymes denotes U/mg protein, the protein concentration based on the procedure of Lowry et al. [40].

## RESULTS

### *Kinetics of Induction of Microsomal and Nuclear Aryl-Hydrocarbon Hydroxylase by 3-Methylcholanthrene*

The kinetics of membrane-bound aryl hydrocarbon hydroxylase induction by 3-methylcholanthrene was studied in all four subcellular particulate fractions (Fig. 1). The 3-methylcholanthrene-induced hydroxylase activity in all fractions was approximately the same at 2, 3 and 5 days after a single large dose of 3-methylcholanthrene; 48 h was therefore selected as the time at which maximal activity and been attained (Fig. 1, bottom). Aryl-hydrocarbon hydroxylase activity reached half-maximal levels by about 8 or 9 h in fraction P and about 21 or 22 h in fraction N.

### *Localization of Activity of Basal and 3-Methylcholanthrene-Induced Aryl Hydrocarbon Hydroxylase*

The hydroxylase and six other membrane-bound activities were examined in five subcellular fractions of liver from control and 3-methylcholanthrene-treated B6 and D2 mice (Tables 1 – 3). 3-Methylcholanthrene-induced activity of microsomal aryl-hydrocarbon hydroxylase reflects one or more forms of cytochrome $P_1$-450, whereas basal hydroxylase activity in control B6 or D2 mice or 3-methylcholanthrene-treated D2 mice represents one or more forms of control monooxygenases mediated by cytochrome *P*-450 [41]. No 3-methylcholanthrene-induced hydroxylase activity was detected in any D2 subcellular fraction (Table 1), yet activity of B6 microsomal aryl-hydrocarbon hydroxylase (fraction P) and of the nuclear hydroxylase (purified fraction N) increased about 4-fold and 15-fold, respectively. The presence of detectable aryl-hydrocarbon hydroxylase (and five of the six other enzymes in Table 1) in the postmicrosomal cytosol (fraction S) is commonly seen for membrane-bound enzyme activities isolated by the method of De Duve et al. [33] and is believed to reflect a small amount of microsomal fragments. 3-Methylcholanthrene treatment had no marked effect on cytochrome *c* oxidase or 5′-nucleotidase activities but decreased significantly glucose-6-phosphatase activity in most fractions of both B6 and D2 mice.

If one examines the subcellular distribution of total activity (Table 2), aryl-hydrocarbon hydroxylase, NADPH : cytochrome *c* reductase, NADH : cytochrome *c* reductase, NADH : cytochrome *c* reductase, and glucose-6-phosphatase were found predominantly in fraction P, cytochrome *c* oxidase predominantly in fraction M, and 5′-nucleotidase predominantly in fraction L. Of interest, the hydroxylase activity in fraction N represented less than 1% of total aryl-hydrocarbon hydroxylase activity. When a microsomal enzyme comprises greater than 99% of total activity in the cell, the possibility of even less than 1% contamination of nuclear membranes with microsomal membranes becomes important.

Does the hydroxylase activity in fraction N therefore represent a distinct moiety or does it represent microsomal contamination? The aryl-hydrocarbon hydroxylase/glucose-6-phosphatase ratio from control

Table 1. *Specific activities of the seven membrane-bound enzymes in various hepatic subcellular fractions of control and 3-methylcholanthrene-treated B6 and D2 mice*
All values are expressed as the average specific activities from three experiments. Coefficients of variance ranged between 0.08 and 0.23. 3-Methylcholanthrene or corn oil alone was given 48 h before killing. Preparation and definition of the particulate fractions N, M, L and P and the supernatant fraction S are described under Materials and Methods

| Enzyme | Subcellular fraction | Specific activity in B6 mice treated | B6 mice control | D2 mice treated | D2 mice control |
|---|---|---|---|---|---|
| | | U/mg protein | | | |
| Aryl-hydrocarbon hydroxylase | N | 310 | 21 | 23 | 25 |
| | M | 820 | 90 | 71 | 68 |
| | L | 1700 | 180 | 190 | 200 |
| | P | 2500 | 610 | 480 | 460 |
| | S | 66 | 1.7 | 1.4 | 1.3 |
| NADPH-cytochrome *c* reductase | N | 9.3 | 9.6 | 7.1 | 7.2 |
| | M | 38 | 43 | 36 | 44 |
| | L | 65 | 57 | 54 | 55 |
| | P | 210 | 160 | 170 | 170 |
| | S | 4.3 | 3.0 | 3.2 | 2.4 |
| NADH-cytochrome *c* reductase | N | 56 | 70 | 69 | 72 |
| | M | 180 | 200 | 200 | 180 |
| | L | 250 | 260 | 300 | 290 |
| | P | 600 | 740 | 820 | 800 |
| | S | 2.7 | 2.8 | 1.3 | 1.3 |
| NADH-cytochrome $b_5$ reductase | N | 110 | 140 | 180 | 200 |
| | M | 670 | 940 | 840 | 910 |
| | L | 610 | 880 | 810 | 820 |
| | P | 1300 | 1700 | 2600 | 2700 |
| | S | 81 | 93 | 81 | 100 |
| Glucose-6-phosphatase | N | 15 | 34 | 17 | 31 |
| | M | 87 | 180 | 140 | 140 |
| | L | 220 | 260 | 220 | 250 |
| | P | 240 | 300 | 220 | 290 |
| | S | 6.0 | 3.9 | 5.2 | 2.7 |
| Cytochrome *c* oxidase | N | 9.7 | 8.4 | 9.8 | 11 |
| | M | 510 | 540 | 560 | 480 |
| | L | 75 | 65 | 57 | 53 |
| | P | 25 | 19 | 26 | 29 |
| | S | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| 5′-Nucleotidase | N | 1.0 | 1.3 | 0.9 | 1.0 |
| | M | 15 | 14 | 26 | 22 |
| | L | 50 | 44 | 58 | 53 |
| | P | 24 | 22 | 26 | 21 |
| | S | 1.9 | 2.4 | 2.9 | 2.6 |

Table 2. *Activity of the seven enzymes in various hepatic subcellular fractions of control and 3-methylcholanthrene-treated B6 and D2 mice*
Data are expressed as a percentage of total cellular activity, 100% being the activity recovered from all five fractions. These results are based on the same samples listed in Table 1 and represent the averages from three experiments

| Enzyme | Subcellular fraction | Enzyme activity in B6 mice treated | B6 mice control | D2 mice treated | D2 mice control |
|---|---|---|---|---|---|
| | | % total | | | |
| Aryl-hydrocarbon hydroxylase | N | 0.6 | 0.3 | 0.5 | 0.3 |
| | M | 7.7 | 5.5 | 7.0 | 4.2 |
| | L | 17 | 15 | 18 | 18 |
| | P | 69 | 78 | 74 | 76 |
| | S | 5.7 | 1.2 | 0.5 | 1.5 |
| NADPH-cytochrome *c* reductase | N | 0.2 | 0.4 | 0.4 | 0.3 |
| | M | 5.0 | 8.6 | 9.0 | 8.3 |
| | L | 8.4 | 13 | 13 | 13 |
| | P | 81 | 72 | 72 | 73 |
| | S | 5.4 | 7.0 | 5.6 | 5.4 |
| NADH-cytochrome *c* reductase | N | 0.5 | 0.7 | 1.0 | 0.7 |
| | M | 8.1 | 9.6 | 9.6 | 8.4 |
| | L | 11 | 14 | 16 | 15 |
| | P | 79 | 75 | 73 | 75 |
| | S | 1.4 | 0.7 | 0.4 | 0.9 |
| NADH-cytochrome $b_5$ reductase | N | 0.4 | 0.4 | 0.5 | 0.4 |
| | M | 11 | 12 | 13 | 10 |
| | L | 10 | 15 | 12 | 11 |
| | P | 69 | 63 | 65 | 69 |
| | S | 9.6 | 9.6 | 9.5 | 9.6 |
| Glucose-6-phosphatase | N | 0.3 | 0.6 | 0.5 | 0.5 |
| | M | 8.3 | 13 | 16 | 14 |
| | L | 22 | 26 | 28 | 24 |
| | P | 67 | 58 | 53 | 59 |
| | S | 2.4 | 2.4 | 2.5 | 2.5 |
| Cytochrome *c* oxidase | N | 0.3 | 0.2 | 0.4 | 0.4 |
| | M | 78 | 79 | 80 | 78 |
| | L | 11 | 11 | 10 | 11 |
| | P | 11 | 9.8 | 9.6 | 10.6 |
| | S | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| 5′-Nucleotidase | N | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| | M | 10 | 10 | 17 | 16 |
| | L | 42 | 49 | 44 | 44 |
| | P | 37 | 27 | 24 | 26 |
| | S | 11 | 14 | 15 | 14 |

B6 and D2 and 3-methylcholanthrene-treated D2 mice (Table 3) ranged between 1.6 and 2.2 in fraction P, and between 0.62 and 1.3 in fraction N. These data indicate that the amount of detectable basal activity of aryl-hydrocarbon hydroxylase in the nuclear fraction of these mice can be completely accounted for by the degree of microsomal membrane contamination (as assessed by the microsomal marker glucose-6-phosphatase). On the other hand, the aryl-hydrocarbon hydroxylase/glucose-6-phosphatase ratio was 10 in fraction P and 21 in fraction N of 3-methylcholanthrene-treated B6 mice; we thus conclude that there is more 3-methylcholanthrene-induced hydroxylase activity in the nuclear fraction of these mice than can be accounted for by the degree of microsomal fragment contribution.

Table 3. *Ratio of specific activity of aryl-hydrocarbon hydroxylase or reductase to glucose-6-phosphatase among the various hepatic subcellular fractions from control and 3-methylcholanthrene-treated B6 and D2 mice*
Values are expressed as the ratios of specific enzyme activities, based on the same samples listed in Table 1

| Enzymes | Sub-cellular fraction | Ratio of specific activities | | | |
|---|---|---|---|---|---|
| | | B6 mice | | D2 mice | |
| | | treated | control | treated | control |
| Aryl hydrocarbon hydroxylase/glucose-6-phosphatase | N | 21 | 0.62 | 1.3 | 0.81 |
| | P | 10 | 2.0 | 2.2 | 1.6 |
| NADPH : cytochrome *c* reductase/glucose-6-phosphatase | N | 0.62 | 0.28 | 0.42 | 0.23 |
| | P | 0.88 | 0.53 | 0.77 | 0.59 |
| NADH : cytochrome *c* reductase/glucose-6-phosphatase | N | 3.7 | 2.1 | 4.1 | 2.3 |
| | P | 2.5 | 4.1 | 3.7 | 2.8 |
| NADH : cytochrome $b_5$ reductase/glucose-6-phosphatase | N | 7.3 | 4.1 | 11 | 6.5 |
| | P | 5.4 | 5.7 | 12 | 9.3 |

### *Localization of Basal and 3-Methylcholanthrene-Inducible Activities of Reductases*

3-Methylcholanthrene given to B6 mice (Table 1) enhanced NADPH : cytochrome *c* reductase activity about 30% in fraction P but depressed NADH : cytochrome *c* and NADH : cytochrome $b_5$ reductase activities by as much as 30% in all subcellular fractions. 3-Methylcholanthrene had no effect on any D2 reductase activity in any of the subcellular fractions.

The total activity of the three reductase in fraction N (Table 2) was always less than 1% of the total cellular reductase activities. The possibility of the smallest amount of microsomal contamination of fraction N is therefore as important with these reductases as that discussed above for aryl hydrocarbon hydroxylase activity.

If one examines the reductase/glucose-6-phosphatase ratios in control and 3-methylcholanthrene-treated B6 and D2 mice (Table 3), the ratios in fraction P were about the same or greater in all cases than the corresponding ratios in fraction N. The amount of detectable basal reductase activities in the nuclear fraction of these mice can thus be completely accounted for by the degree of microsomal contribution (as assessed by the glucose-6-phosphatase marker). In 3-methylcholanthrene-treated B6 mice, the NADH : cytochrome *c* reductase/glucose-6-phosphatase and the NADH : cytochrome $b_5$ reductase/glucose-6-phosphatase ratios in fraction N tended to be higher than those in fraction P. It would appear that more of these two reductase activities exist in the nuclear fraction of these mice than can be accounted for by the degree of microsomal fragment contribution. Because 3-methylcholanthrene treatment diminishes glucose-6-phosphatase specific activity (Table 1), however, these small increases in the ratios may reflect microsomal membrane changes due to glucose-6-phosphatase depletion and/or errors in the glucose-6-phosphatase assay, which can be considerable at very low enzyme activities.

## DISCUSSION

In light of the data shown in this present report, one must consider seriously the possibility of microsomal monooxygenase activities contributing all, or most, to what has been considered heretofore as enzyme activities from 'highly pure' nuclear fractions [1–31]. The same is true for immunological studies [8,11–13]. We have shown here that the nuclear envelope contributes less than 1% to total aryl hydrocarbon hydroxylase activity in the liver cell of 3-methylcholanthrene-treated B6 mice and that the amount of detectable basal aryl-hydrocarbon hydroxylase activity in the nuclear fraction of control (or 3-methylcholanthrene-treated genetically nonresponsive) mice can be wholly accounted for by the degree of contamination by microsomal membrane fragments.

The *Ah* locus regulates the induction by 3-methylcholanthrene and certain other polycyclic aromatic compounds of numerous drug-metabolizing enzyme activities such as the aryl hydrocarbon hydroxylase associated with cytochrome $P_1$-450 (reviewed in [41]). A cytosolic receptor highly specific for these inducers appears to be essential for the induction process; B6 mouse liver has at least 50-times more receptor than D2 mouse liver [42]. The expression of 3-methylcholanthrene-inducible hydroxylase in nuclear membranes (Table 1), like that in microsomal membranes, thus appears to be controlled by the *Ah* regulatory gene. The slower induction kinetics of nuclear aryl hydrocarbon hydroxylase activity (Fig. 1), compared with that of the microsomal hydroxylase activity, may represent the transition of newly synthesized enzyme

from the endoplasmic reticulum to the nuclear membranes.

It is attractive to consider that nuclear hydrocarbon hydroxylase activity, even though less than 1% of the total cellular activity, contributes to the formation of most short-lived metabolic intermediates that bind covalently to intranuclear DNA and are presumably related to chemical mutagenesis and carcinogenesis [14,16,24]. Estimates of a half-life for the various stereo-isomers of benzo(*a*)pyrene 7,8-dihydrodiol-9,10-epoxide, for example, are extremely difficult to determine, however, and depend on ionic strength, pH, and types of proteins and lipids present in the experimental test solution; under physiological conditions, the diol-epoxides may therefore react in less than 1 ms or longer than 15 min [43,44]. Certainly the latter estimate allows more than ample time for reactive intermediates generated by the microsomal hydroxylase to bind covalently to nuclear DNA in the same cell or even in cells of some distant organ in the animal. Contrary to common belief, oxygenated benzo(*a*)pyrene intermediates pass readily through cellular or subcellular membranes. Evidence in support of this concept has been shown: radiolabeled benzo(*a*)pyrene 7,8-dihydrodiol is taken up by cells in culture more quickly than benzo(*a*)pyrene and also is metabolized more rapidly to more polar excreted (presumably conjugated) products [45]; intraperitoneally injected benzo(*a*)pyrene 7,8-dihydrodiol-9,10-epoxide in newborn Swiss-Webster mice is at least 150-times more effective than benzo(*a*)pyrene in causing pulmonary and lymphatic tumors [46].

In summary, we have shown that 3-methylcholanthrene-induced aryl-hydrocarbon hydroxylase activity exists in nuclear membranes at levels greater than can be accounted for by liver microsomal contamination. This is not true for the hydroxylase activity in control mice or for any of three NAD(P)H-reductase activities. The possible role of 3-methylcholanthrene-induced nuclear activity in the mechanism of chemical carcinogenesis remains to be determined.

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## REFERENCES

1. Kasper, C. B. (1971) *J. Biol. Chem. 246*, 577–581.
2. Khandwala, A. S. & Kasper, C. B. (1973) *Biochem. Biophys. Res. Commun. 54*, 1241–1246.
3. Rogan, E. & Cavalieri, E. (1978) *Mol. Pharmacol. 14*, 215–219.
4. Fahl, W. E., Jefcoate, C. R. & Kasper, C. B. (1978) *J. Biol. Chem. 253*, 3106–3113.
5. Vizethum, W. & Goerz, G. (1979) *Chem.-Biol. Interact. 28*, 291–299.
6. Watanabe, M., Ariji, F., Takusagawa, K. & Konno, K. (1975) *Gann, 66*, 399–409.
7. Bresnick, E., Stoming, T. A., Vaught, J. B., Thakker, D. R. & Jerina, D. M. (1977) *Arch. Biochem. Biophys. 183*, 31–37.
8. Sagara, Y., Harano, T. & Omura, T. (1978) *J. Biochem. (Tokyo) 83*, 807–812.
9. Vaught, J. B., Klohs, W. & Gurtoo, H. L. (1978) *Life Sci. 21*, 1497–1504.
10. Viviani, A., Lutz, W. K. & Schlatter, C. (1978) *Biochem. Pharmacol. 27*, 2103–2108.
11. Thomas, P. E., Korzeniowski, D., Bresnick, E., Bornstein, W. A., Kasper, C. B., Fahl, W. E., Jefcoate, C. R. & Levin, W. (1979) *Arch. Biochem. Biophys. 192*, 22–26.
12. Bresnick, E., Boraker, D., Hassuk, B., Levin, W. & Thomas, P. E. (1979) *Mol. Pharmacol. 16*, 324–331.
13. Guengerich, F. P. (1979) *Biochem. Pharmacol. 28*, 2883–2890.
14. Bresnick, E. (1978) *Advances in Enzyme Regulation* (Weber, G., ed.) pp. 347–361, Pergamon Press, Oxford and New York.
15. Mukhtar, H., Elmamlouk, T. H. & Bend, J. R. (1979) *Arch. Biochem. Biophys. 192*, 10–21.
16. Yang, C. S. & Pezzuto, J. M. (1979) *Polynuclear Aromatic Hydrocarbons* (Jones, P. W. & Leber, P., eds) pp. 653–667, Ann Arbor Science Publishers, Michigan.
17. Vaught, J. & Bresnick, E. (1976) *Biochem. Biophys. Res. Commun. 69*, 587–591.
18. Pezzuto, J. M., Lea, M. A. & Yang, C. S. (1976) *Cancer Res. 36*, 3647–3653.
19. Jernström, B., Vadi, H. & Orrenius, S. (1976) *Cancer Res. 36*, 4107–4113.
20. Pezzuto, J. M., Lea, M. A. & Yang, C. S. (1977) *Cancer Res. 37*, 3427–3433.
21. Bresnick, E., Vaught, J. B., Chuang, A. H. L., Stoming, T. A., Bockman, D. & Mukhtar, H. (1977) *Arch. Biochem. Biophys. 181*, 257–269.
22. Pezzuto, J. M., Yang, C. S., Yang, S. K., McCourt, D. W. & Gelboin, H. V. (1978) *Cancer Res. 38*, 1241–1245.
23. Viviani, A. & Lutz, W. K. (1978) *Cancer Res. 38*, 4640–4644.
24. Guenthner, T. M., Jernström, B. & Orrenius, S. (1979) *Biochem. Biophys. Res. Commun. 91*, 842–848.
25. Jennette, K. W., Bornstein, W., Chuang, A. H. L. & Bresnick, E. (1979) *Biochem. Pharmacol. 28*, 338–339.
26. Sakai, S., Reinhold, C. E., Wirth, P. J. & Thorgeirsson, S. S. (1978) *Cancer Res. 38*, 2058–2067.
27. Kawajiri, K., Yonekawa, H., Hara, E. & Tagashira, Y. (1979) *Cancer Res. 39*, 1089–1093.
28. Stout, D. L. & Becker, F. F. (1979) *Cancer Res. 39*, 1168–1173.
29. Tierney, B., Bresnick, E., Sims, P. & Grover, P. L. (1979) *Biochem. Pharmacol. 28*, 2607–2610.
30. Diaz-Gomez, M. I. & Castro, J. A. (1980) *Res. Commun. Chem. Pathol. Pharmacol. 27*, 191–194.
31. Gazzotti, G., Garattini, E. & Salmona, M. (1980) *Chem.-Biol. Interact. 29*, 189–195.
32. Nebert, D. W. (1964) Thesis, University of Oregon Medical School.
33. De Duve, C., Pressman, B. C., Gianetto, R., Wattiaux, R. & Appelmano, F. (1955) *Biochem. J. 60*, 604–617.
34. Blobel, G. & Potter, V. R. (1967) *Science (Wash. DC) 154*, 1662–1665.
35. Nebert, D. W. (1978) *Methods Enzymol. 52*, 226–240.
36. Mackler, B. & Green, D. E. (1956) *Biochim. Biophys. Acta, 21*, 1–13.
37. Strittmatter, P. (1967) *Methods Enzymol. 10*, 561–565.
38. Eylar, E. H. & Hagopian, A. (1971) *Methods Enzymol. 22*, 138–143.
39. Cooperstein, S. J. & Lazarow, A. (1951) *J. Biol. Chem. 189*, 665–670.

40. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.
41. Nebert, D. W. & Jensen, N. M. (1979) *CRC Crit. Rev. Biochem. 6*, 401–437.
42. Okey, A. B., Bondy, G. T., Mason, M. E., Kahl, G. F., Eisen, H. J., Guenthner, T. M. & Nebert, D. W. (1979) *J. Biol. Chem. 254*, 11 636–11 648.
43. Yang, S. K., McCourt, D. W., Leutz, J. C. & Gelboin, H. V. (1977) *Science (Wash. DC) 196*, 1199–1201.
44. Whalen, D. L., Ross, A. M., Yagi, H., Karle, J. M. & Jerina, D. M. (1978) *J. Am. Chem. Soc. 100*, 5218–5221.
45. Kano, I., Gielen, J. E., Yagi, H., Jerina, D. M. & Nebert, D. W. (1977) *Mol. Pharmacol. 13*, 1181–1186.
46. Buening, M. K., Wislocki, P. G., Levin, W., Yagi, H., Thakker, D. R., Akagi, H., Koreeda, M., Jerina, D. M. & Conney, A. H. (1978) *Proc. Natl Acad. Sci. USA 75*, 5358–5361.

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