Nuclear Uptake and Subsequent Nuclear Metabolism of Benzo(a)pyrene Complexed to Cytosolic Proteins

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ABSTRACT

The binding of [3H]benzo(a)pyrene to proteins of rat liver cytosol, the nuclear uptake of cytosolic protein-bound [3H]benzo(a)pyrene, and the subsequent nuclear metabolism of the polycyclic hydrocarbon were investigated. The binding of [3H]benzo(a)pyrene to cytosol had a saturable high affinity component with a Kd of 2.5 μM and a capacity of 530 fmol/mg protein. Specific binding of [3H]benzo(a)pyrene to cytosol was also assayed using sucrose density gradient analysis. Nuclear uptake of protein-bound [3H]benzo(a)pyrene was demonstrated both directly and by sucrose density gradient analysis. The nuclear benzo(a)pyrene was readily converted to metabolites which were qualitatively and quantitatively no different from nuclear metabolites of exogenously (not protein associated) added [3H]benzo(a)pyrene.

INTRODUCTION

The presence of cytosolic proteins having high affinity for inducers of cytochrome P-450c and cytochrome P-450c-mediated AHH has been the subject of several investigations aimed toward elucidating mechanisms of induction. Earlier work had shown that the cytosol from homogenates of mouse skin epidermis bound MC noncovalently in vitro (38); furthermore, after in vivo topical application (37), MC became bound. When the inducer, MC, was used as ligand, binding protein fractions were detected in rat liver cytosol (40) and cultured rat hepatocytes (15) which possessed high affinity for MC; the translocation of MC to the nucleus was demonstrated. The protein:ligand complex(es) also possessed the ability to bind to DNA:cellulose, and the presence or absence of these DNA binding complexes correlated with the inducibility of the cytochrome P-450c monoxygenase system in C57BL/6J and DBA/2J mice (42).

The potent inducer of AHH, TCDD, binds very avidly and noncovalently to a cytosolic binding protein fraction in mouse liver (28, 31) and in cultured cell lines (14). The TCDD:protein complex is thought to function in the specific nuclear uptake of TCDD (12, 28). The levels of this binding protein in a variety of inducible mouse strains (28), but not in the cultured cell lines (14), correlated with the level of elevation of AHH. The levels of TCDD:cytosolic binding proteins were decreased; while the activity of microsomal 7-ethoxycoumarin O-deethylase was increased (12) after treatment with the inducers, β-naphthoflavone (28) or TCDD (12). Treatment of cell lines with the inducers, benzo(a)anthracene or MC, resulted in elevated binding protein levels in inducible, but not in noninducible, lines (14). The segregation of TCDD toxicity with the Ah locus in C57BL and DBA mice and their progeny indicated that the toxicity of TCDD and its congeners may be mediated through binding of these compounds to the cytosolic binding protein (30).

BP, another inducer of cytochrome P-450c, is an effective competitor for the binding of MC (40) and TCDD (28) to hepatic cytosolic proteins. Its binding to various subcellular liver fractions has been reported in rat liver using a dextran charcoal assay technique (19). The levels of binding protein in cytosol were inducible with BP (19).

Studies with steroid hormones have provided a model (7, 11, 21) for the cellular action of low molecular weight cytochrome P-450c inducers. After entry into the cell, the inducer interacts with a cytosolic receptor, and subsequently, this complex is transferred into the nucleus. Specific mRNA synthesis and subsequent protein synthesis are the manifestations of the interaction of the protein-bound ligand or the ligand itself with nuclear components. Specifically in regard to the binding protein, it is conceivable that the polycyclic hydrocarbon may undergo nuclear metabolism to agents which are directly involved in the induction process. The current investigation was undertaken with BP to determine whether cytosolic protein-bound BP could be metabolized by nuclear cytochrome P-450 and the type and quantity of such metabolites, if formed.

MATERIALS AND METHODS

Chemicals. [3H]BP (40 or 65 Ci/mmol), NCS tissue solubilizer, and PCS were purchased from American Chemical Co., Pittsburgh, Pa.; dextran and other chemicals were purchased from Sigma Chemical Co., St. Louis, Mo.

Animals. Male Sprague-Dawley rats (100 to 125 g) obtained from Charles River Breeding Laboratories, Inc., were used as the source of liver.

Preparation of Tissue Fractions. Cytosols containing 20 to 25 mg protein per ml were prepared from livers perfused with HEDG buffer, and the livers were then homogenized using a ratio of 1 g liver to 3 ml HEDG buffer as described (40). After centrifugation, the cytosol was diluted with HEDG to give final protein concentrations of 1 or 4.5 mg/ml. Nuclei were prepared from rat liver as described (25) and suspended in a solution (Sucrose A) of 0.34 M sucrose:0.25 M spermidine diphosphate:15 mM magnesium acetate:0.5 mM phenylmethanesulfon fluoride:0.5 mM diithiothreitol at a protein concentration of 4 to 13 mg/ml. The protein concentration in cytosol and nuclear suspensions was determined by the method of Lowry et al. (26) using BSA as the reference standard.
Specific Binding of $[^{3}H]BP$. $[^{3}H]BP$ (0.3 to 22 pmol) dissolved in 0.01 ml dimethyl sulfoxide was added to the liver cytosol containing 1.0 or 4.5 mg protein per ml. In parallel experiments, $[^{3}H]BP$ (0.3 to 22 pmol) with a 160-fold excess of unlabeled hydrocarbon was used. The BP: liver cytosol mixtures were incubated at 0-4° for 80 min, and then, 0.8-ml aliquots were vortexed for 8 sec with a pellet of dextran-coated charcoal obtained by centrifuging in 1.5-ml Microfuge tubes 1.0 ml of a freshly prepared 7.5 or 10 mg suspension of charcoal in HEDG containing 0.75 or 1.0 mg dextran. Adsorption of free BP was allowed to proceed for 30 min at 0-4°, with vortexing at 10-min intervals, and after 30 min, the tubes were centrifuged at 8000 x g for 1.5 min. The radioactivities in 0.2-ml aliquots of the supernatant fractions were determined, and the specific binding was taken to be the difference between the radioactivities remaining in the cytosol after incubation of $[^{3}H]BP$ with and without the 160-fold excess of unlabeled hydrocarbon (31). Duplicates, both with and without unlabeled hydrocarbon, were used unless otherwise stated. Neutral Norit, alkaline Norit, and Nuchar C carbon gave similar values for the specific binding, although neutral Norit was routinely used in the studies reported herein.

Sucrose Density Gradient Studies. Linear gradients of 5 to 20% sucrose in approximately 4 to 4.5 ml, which contained HEDG buffer, were prepared at 0-4° and allowed to equilibrate for at least 1 hr before application of a 0.5-ml sample. Some gradients were prepared over a 0.4-ml cushion of 2.3 M sucrose. Centrifugation was conducted at 189,000 x g (average) for 16 hr at 4° in a Beckman SW 50.1 rotor, and then 15-drop fractions were collected directly into scintillation vials using a tube-piercing unit. The remaining pellet was assayed for radioactivity after removal of the end of the centrifuge tube. In some experiments, [14C]BSA with a sedimentation of 4.4S was added as an internal molecular size marker.

Nuclear Uptake of Radioactivity from $[^{3}H]BP$:Cytosol Complex. $[^{3}H]BP$:cytosol complex was prepared as described above by incubating $[^{3}H]BP$ (3.13 nm) with cytosol (4.5 mg protein per ml) and centrifuging at 105,000 x g for 45 min. The complex (1.0 ml) was added to nuclei (0.36 to 19.4 mg protein) which were suspended in 1.6 ml Sucrose A solution. After incubation for 15 min at 37°, the nuclei were separated from the cytosol by centrifugation at 800 x g for 5 min at 1-4°, and the nuclear pellets were washed twice by resuspension and centrifugation in a mixture of 1.6 ml Sucrose A:1.0 ml HEDG. The nuclear pellets were digested in 1.0 ml NCS before determination of radioactivity. The incubation medium containing the $[^{3}H]BP$-complex which had not been taken up by the nuclei was also counted.

Metabolism of $[^{3}H]BP$ Taken Up by Nuclei. $[^{3}H]BP$:cytosol complex was prepared using 2.2 nm $[^{3}H]BP$ and cytosol (4.5 mg protein per ml) that had been dialyzed against 50 volumes of HEDG buffer for 24 hr before dilution. The dextran charcoal-treated supernatant fraction was re centrifuged for 45 min at 105,000 x g at 4°. A nuclear suspension of 0.8 ml which contained 11.1 mg of protein was added to 1.0 ml of the $[^{3}H]BP$:cytosol complex or 1.0 ml of cytosol alone and incubated at 37° for 10 min. The nuclear pellets were isolated as described above, and 1.0 ml fresh cytosol or HEDG buffer and 0.8 ml Sucrose A were added. To nuclei that had not been exposed to the $[^{3}H]BP$:cytosol complex, $[^{3}H]BP$ in 0.01 ml dimethyl sulfoxide was added to give a BP concentration of 0.6 nm. The metabolic reaction was started by addition of 0.3 mNADPH in a final volume of 2.0 ml, and the incubation was conducted at 37° for 10 min. Nuclear and supernatant fractions were separated, the nuclei were washed in Sucrose A:HEDG, and the radioactivity and the metabolites in each fraction were determined. The organosoluble metabolites from nuclei were extracted with 1.0 ml acetonitrile and 2.0 ml ethyl acetate, while the supernatant was extracted with 1.5 ml acetonitrile and 3.0 ml ethyl acetate. Each fraction was reextracted once with ethyl acetate; additional extractions removed no further counts from these fractions. After addition of marker compounds, organosoluble metabolites were analyzed by high-pressure liquid chromatography on a Waters C$_{18}$Bondapak column using a methanol:water gradient protocol consisting of 5 min at 50%, 20-min linear at 50 to 70%, 13 min at 70%, 22-min linear at 70 to 95%, and 15 min at 95%. The marker compounds included: trans-BP 9-, 10-, 4-, 5-, and 7,8-dihydrodiols; BP 1,6-, 3,6-, and 6,12-quinones; BP 3-phenol and BP 9-phenol; and BP itself. They emerged from the column in the stated order with trans-BP,9,10-dihydrodiol eluting the fastest. All radioactivities were determined by liquid scintillation counting in 10 ml Triton X-100 cocktail or in 4.0 ml PCS:xylene (2:1 by volume).

RESULTS

Measurement of Specific Binding of $[^{3}H]BP$ to Cytosol

Dextran Charcoal Adsorption. The measurement of specific binding of polycyclic hydrocarbons and TCDD to hepatic cytosolic proteins has met with varied success when charcoal dextran (widely used in steroid receptor assays (3, 24, 27, 33)) has been used to separate the protein-bound ligand from the free ligand. Earlier papers (14, 19, 31) reported satisfactory results, while some difficulties have been noted in more recent studies (8, 28, 40). In order to understand these discrepancies, we examined some conditions of the assay in some detail. The level of binding of $[^{3}H]BP$ to the proteins in cytosol both in the presence and absence of excess unlabeled BP was found to exhibit a marked dependence upon the time of treatment with dextran charcoal (Chart 1A) and upon the quantity of dextran charcoal used (Chart 1B). The level of specific binding, i.e., the saturable binding, however, showed little dependence on these variables. When the concentration of unlabeled competitor was increased, the radioactivity remaining in the aqueous phase after dextran charcoal treatment, i.e., protein-bound BP, decreased to a minimum at an 80-fold excess and then decreased to a value exceeding that obtained when only labeled BP was used (Chart 2). The calculated specific binding was maximal at 80-fold excess of unlabeled BP. When the adsorptions of $[^{3}H]BP$ from HEDG (no cytosol) at the same ratios were determined, about 3.5 to 4% of the radioactivity remained in solution after dextran charcoal treatment, until the capacity of the adsorbent became limiting above a 200-fold excess. At the highest ratios of unlabeled to labeled BP, the hydrocarbon was...
Chart 2. Influence of unlabeled BP on the total binding of [3H]BP by rat liver cytosol. Cytosol (1.0 mg protein per ml) was reacted with [3H]BP (3.98 nm) for 80 min at 0° in the presence of increasing additions of unlabeled BP. Binding was measured using dextran (0.75 mg)-coated charcoal (7.5 mg) per 0.8 ml for 30 min at 0-4°. Total binding (O), apparent specific binding (•), and apparent binding in buffer blanks (x) are shown.

not soluble in buffer in the absence of protein. We have chosen to work at a concentration of 160-fold excess of unlabeled BP, which results in a slight loss in specific binding (10%) but which should result in all specific binding sites being blocked. A dextran charcoal treatment time of 30 min and dextran:charcoal ratio of 0.75 and 7.5 mg, respectively, per ml cytosol were adopted from the other experiments. When the concentration of [3H]BP was varied from 0.3 to 22 nM and the binding was measured using dextran charcoal in the presence and absence of excess unlabeled compound, the specific binding data for [3H]BP presented in Chart 3 were obtained. At higher concentrations of [3H]BP, failure of the dextran charcoal to adsorb all the unbound polycyclic hydrocarbon when excess material was present resulted in an upward curvature of the nonspecific binding curve. The data from BP concentrations of 0.3 to 14.0 nM were replotted according to the method of Scatchard (35) (Chart 4), and a Kd of 2.54 nM and a binding capacity of 530 fmol/mg cytosolic protein were calculated. These values are in close agreement with 2.78 nM and 770 fmol/mg obtained for MC by chromatography on Sepharyl S-200 columns (40). The use of dextran charcoal to measure specific binding for polycyclic hydrocarbons was also justified by comparisons with results obtained using sucrose density gradients.

Sucrose Density Gradient Studies. When [3H]BP was incubated with cytosol in the presence and absence of an excess of BP for 90 min at 0-4° (no charcoal dextran treatment) and when the resultant solutions were examined after sucrose density gradient centrifugation, specific binding to a fraction which sedimented at Fraction 8 was detected (Chart 5A). Values for the specific binding of BP to cytosolic protein obtained by this method and by dextran-coated charcoal were higher than when calculated from sucrose density gradient centrifugation data after a dextran-binding step (Table 1; Chart 5B). Lower values for the specific binding obtained by the latter combined methods occurred because of partitioning of bound [3H]BP into the polyallomer walls of the centrifuge tube. A fraction in excess of 20% of the applied radioactivity was always associated with the tube after centrifugation.

When [14C]BSA was included as a molecular size marker, the peak of protein-bound BP, Fraction 8, cosedimented with the BSA, i.e., had a sedimentation of 4.4S. Plasma and cytosol at the same protein concentration were compared in terms of their complexation of BP (Chart 6). With plasma, after dextran charcoal treatment, the amount of protein-bound BP which occurred in the peak at Fractions 8 to 9 was only 25% of that seen with cytosol; the heavier peak at Fractions 16 to 17 was completely absent in the case of plasma. Since the plasma proteins present as contaminants in the perfused liver cytosol are present in concentrations less than those utilized in the plasma experiments mentioned above, the binding of [3H]BP in the liver preparations must be preponderantly due to hepatic cytosolic proteins. The faster sedimenting region in cytosol did not always appear as a large discrete peak on sucrose gradients and was often present as only a small peak or shoulder.

Nuclear Translocation of BP

Efforts have not been successful in detecting nuclear translocation of [3H]BP by incubation of freshly prepared nuclei with cytosol that had been treated with BP followed by washing of the nuclei with 0.5 to 1% Triton X-100 in 100 mM KCl:25 mM Tris:5 mM MgCl₂:1 mM diethiothreitol buffer (12). When cytosol was incubated with [3H]BP, treated with dextran charcoal (charged cytosol), and subsequently incubated with hepatic...
nuclei for 1 hr at 0–4°, translocation of BP into the nuclei could be detected by sucrose density gradient centrifugation (Chart 7). Comparison of the BP-charged cytosol that had been incubated in the presence of nuclei with BP-charged cytosol having no nuclei added and that was kept on ice for an additional hr before centrifugation on sucrose gradients showed a diminution in the radioactivity under the peak at Fractions 8 to 9 and a complete disappearance of the second peak at Fraction 13 in the nuclei-treated BP-charged cytosol. Concurrently, an increase in radioactivity was found within the 2.3 M sucrose cushion and the pellet. Inclusion in the cytosol incubation of an excess of unlabeled BP or MC very substantially reduced the height of the lighter peak and completely eliminated the heavier (data not shown). When the nuclear uptake experiment was conducted in cytosol charged in the presence and absence of an excess of unlabeled hydrocarbon, a greater percentage of radioactivity (of total placed on gradient) was observed in nuclei that had been incubated with cytosol charged in the absence of unlabeled competitor hydrocarbon, i.e., 22 versus 15%.

Further demonstration of nuclear uptake of \([^{3}H]BP\)-charged cytosol was obtained by incubation with increasing numbers of nuclei (Chart 8) and their subsequent separation. This uptake reached a value greater than 80% at a nuclear:cytosolic protein ratio of 4.3 and was paralleled by a decrease in the cytosolic radioactivity. Incubation of BP-charged cytosol at 37° for 15 min followed by a second dextran charcoal treatment resulted in a 40% loss of specifically bound BP when compared to a control that had been kept at 0–4°. A similar loss was seen in a sample which was frozen and stored overnight at −20°. The nuclear uptake at 37° is therefore composed of both translocated, i.e., protein-associated hydrocarbon, and up to 32% of hydrocarbon which partitioned into the nuclei after dissociation of the BP:cytosolic protein complex.

Table 1

| Nature of binding | Dextran charcoal treatment alone | No dextran charcoal treatment | After dextran charcoal treatment |
|-------------------|---------------------------------|-------------------------------|---------------------------------
| Specific + nonspecific | 0.284 ± 0.047 (3) | 0.576 (2) | 0.150 ± 0.083 (3) |
| Nonspecific | 0.080 ± 0.019 (3) | 0.280 (2) | 0.016 ± 0.005 (3) |
| Specific | 0.224 ± 0.031 (3) | 0.317 (2) | 0.134 ± 0.082 (3) |

*a* Mean ± S.D.

*b* Numbers in parentheses, number of experiments.

Chart 5. Sucrose density gradient analysis of \([^{3}H]BP\)-treated cytosol with (B) and without (A) dextran charcoal treatment. Cytosol (4.5 mg protein per ml) was treated with \([^{3}H]BP\) (3.13 nM) in the absence (C) or presence (D) of a 160-fold excess of unlabeled BP at 0–4° for 90 min and either had no treatment (A) or was subjected to dextran:charcoal (1:10 mg) treatment per ml cytosol for 15 min at 0° (B). Samples (0.5 ml) were applied to gradients (4.5 ml) of 5 to 20% sucrose in HEDG buffer, and the samples were centrifuged at 189,000 x g (average) for 16 hr.

Chart 6. Comparison of binding of \([^{3}H]BP\) to rat plasma and cytosol from perfused rat livers. Cytosol (4.5 mg protein per ml) (C) or heparinized plasma (4.5 mg protein per ml) (D) was treated at 0–4° for 80 min with \([^{3}H]BP\) (3.5 nM) and with dextran charcoal, and 0.5-ml fractions were analyzed on 4.5 ml 5 to 20% sucrose gradients by centrifugation for 16 hr at 189,000 x g (average). Fractions (0.2 ml) were collected using an ISCO Model 640 density gradient fractionator.
Metabolites produced by nuclei from the \([3H]BP\) taken up during a prior incubation at 37° with charged cytosol ("hot" nuclei) were compared with those obtained using preincubated nuclei that had not been exposed to BP until the metabolic incubation. The effect of addition of untreated cytosol to hot nuclei before the metabolism was also examined. The distribution of radioactivity (Table 2) showed that addition of NADPH was required for nuclear metabolism; virtually no nuclear covalently bound or organoextractable metabolic radioactivity was seen in its absence (Table 2, Experiment 1). With addition of cofactor, extensive metabolism occurred (Table 2). Additionally, virtually all of the supernatant radioactivity was nonextractable from the aqueous phase. Few differences were observed in BP metabolism in the systems in which BP was taken up from charged cytosol (Table 2, Experiment 3) or was introduced at the beginning of the metabolism (Table 2, Experiment 4). However, the presence of cytosol (Table 2, Experiment 2) did have a marked effect. The amount of metabolites found in the nucleus was reduced, and that found in the incubation supernatant was increased. Some of the metabolism may have arisen through the metabolic ability of cytosol (Table 2, Experiment 5).

The metabolism was initiated by addition of NADPH (0.3 mM) and conducted for 10 min at 37° under air. The nuclei were separated from the supernatant by centrifugation, washed with sucrose A,HEDG, and worked up in parallel with the supernatant. The hot nuclei contained 74% of the radioactivity added in Experiments 4 and 5 were equivalent to that present in Experiments 1 to 3. Data shown are of a representative experiment.

### Table 2

<table>
<thead>
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<th></th>
<th>Nuclei</th>
<th>Supernatant</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>NADPH</td>
<td>Total</td>
</tr>
<tr>
<td>1. Hot nuclei + buffer</td>
<td>-</td>
<td>67.4</td>
</tr>
<tr>
<td>2. Hot nuclei + cytosol</td>
<td>+</td>
<td>31.9</td>
</tr>
<tr>
<td>3. Hot nuclei + buffer</td>
<td>+</td>
<td>49.4</td>
</tr>
<tr>
<td>4. Unlabeled nuclei + buffer + ([3H]BP)</td>
<td>+</td>
<td>56.3</td>
</tr>
<tr>
<td>5. No nuclei + cytosol + ([3H]BP)</td>
<td>+</td>
<td>67.7</td>
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</tbody>
</table>

* Results are expressed as a percentage of the radioactivity in the metabolism incubation.

* This represents the radioactivity present in the ethyl acetate extract which was eluted by the high-pressure liquid chromatography before BP, expressed as a fraction of the total present in the metabolism incubation.

* ND, not determined.
metabolism with hot nuclei (Table 3, Experiment 3) and metabolism of BP introduced exogenously immediately before the metabolism incubation (Table 3, Experiment 4).

**DISCUSSION**

The use of dextran charcoal apparently is satisfactory for measurement of the binding of BP to protein in liver cytosol. Difficulties experienced by some investigators and ourselves (8, 28, 40) have perhaps been due to the rather critical limits imposed on the excess unlabeled ligand present and the amount of dextran charcoal used. Levels of the binding proteins found for BP in the present work are about 10-fold higher than levels determined for TCDD in mouse liver preparations (28, 31) and for BP in rat liver preparations (19), but they agree with those found in rat liver (40) and a rat liver cell line with MC as the ligand. In our hands, the dextran charcoal treatment resulted in a small reduction in protein concentration (approximately 10%); therefore, errors may be introduced by adsorption of the protein:ligand complex. One difference between our work and that of Hommes and Eller (19) lies in their dextran charcoal pretreatment of the cytosol before incubation with BP. Comparison of dextran charcoal with sucrose density gradient analysis (Table 1) gave similar results and supports the use of the former more convenient technique; the same conclusions were reached using MC as ligand (39). Good correlations between these techniques have also been found with estrogen and progesterone receptors in humans (1). In the data reported here, sucrose gradient analysis resulted in higher levels of both nonspecific and total binding. When both techniques were used sequentially, levels found for BP binding approached those published for TCDD (28, 31).

The irregular appearance in sucrose gradients of a heavy, faster sedimenting region after reaction of BP with cytosol and depletion studies. Nuclear translocation is well known in steroid studies (7, 11, 21) and has been reported when MC (40), BP (19), and TCDD (12, 28, 29) have been used as ligands. More recent evidence for TCDD (28, 29) and MC (15) has indicated that the intact ligand-receptor complex may be extracted from the nucleus. This is therefore strong evidence for the translocation of the intact ligand:protein complex. Our evidence suggests, however, that some of the hydrocarbon:protein complex may break down on incubation with nuclei and therefore be bound to the nuclei as "free" BP. On sucrose gradient analysis, where the BP sedimenting at the regions of 4 to 5S and 8 to 9S would be expected to be protein associated, comparison of dextran charcoal with sucrose density gradient analysis (Table 1) gave similar results and supports the use of the former more convenient technique; the same conclusions were reached using MC as ligand (39). Good correlations between these techniques have also been found with estrogen and progesterone receptors in humans (1). In the data reported here, sucrose gradient analysis resulted in higher levels of both nonspecific and total binding. When both techniques were used sequentially, levels found for BP binding approached those published for TCDD (28, 31).

The irregular appearance in sucrose gradients of a heavy, faster sedimenting region after reaction of BP with cytosol could not be explained and is under further investigation. In studies with MC (39) and TCDD (28) as ligands, the occurrence of such a peak depended upon the absence of salt and the presence of glycerol. Such a peak has also been reported with lung preparations (41). With TCDD and mouse liver preparations, a heavier sedimenting region, which was substantially reduced in the presence of unlabeled BP, was seen indicating that BP binds to a protein sedimenting in that region (28). However, [3H]BP gave rise to only one prominent peak in the 4.4S region with only little binding in the heavier region. This apparent contradiction is as yet unexplained. It is not known whether the heavier peaks seen in the present work for BP and for MC correspond to that seen for TCDD (28). We have found here that binding to plasma proteins cannot account for the radioactivity sedimenting at 4.4S in [3H]BP-treated liver cytosols.

Evidence for translocation of BP:cytosol complex into hepatic nuclei was obtained from both sucrose density gradients and depletion studies. Nuclear translocation is well known in steroid studies (7, 11, 21) and has been reported when MC (40), BP (19), and TCDD (12, 28, 29) have been used as ligands. More recent evidence for TCDD (28, 29) and MC (15) has indicated that the intact ligand-receptor complex may be extracted from the nucleus. This is therefore strong evidence for the translocation of the intact ligand:protein complex. Our evidence suggests, however, that some of the hydrocarbon:protein complex may break down on incubation with nuclei and therefore be bound to the nuclei as "free" BP. On sucrose gradient analysis, where the BP sedimenting at the regions of 4 to 5S and 8 to 9S would be expected to be protein associated, incubation with nuclei resulted in the complete removal of the heavier sedimenting species and a partial decrease (45%) of the 4 to 5S species. This was accompanied by an increase of radioactivity in the nuclear pellet.

In metabolism experiments, the results showed no differences between the profiles seen with translocated BP and BP freshly added to the incubation medium in the presence of buffer. Nuclear metabolism in the presence of fresh cytosol showed differences which could only be ascribed to the latter. Less radioactivity was bound to the nuclei, and a greater fraction of metabolites was found in the supernatant. Cytosol reduced the formation of BP:DNA adducts in microsomal and nuclear activation systems (13, 16) partly through the activity of glutathione S-transferase B. The profiles of the organosoluble metabolites from the nuclei showed that the 7,8-dihydrodiol of BP, a proximate carcinogen (23), to be the predominant dihydrodiol formed and that phenols chromatographing with 9-hydroxybenzo(a)pyrene were formed in amounts equal to or greater than phenols which chromatographed with the 3-hydroxy derivative. No qualitative differences between these pro-

**Table 3**

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<th>Detail</th>
<th>NADPH</th>
<th>% metabolized</th>
<th>Dihydrodiols&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Quinones&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Phenols&lt;sup&gt;b&lt;/sup&gt;</th>
<th>% of recovery&lt;sup&gt;c&lt;/sup&gt;</th>
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<tr>
<td>1. Hot nuclei + buffer</td>
<td>+</td>
<td>2.5</td>
<td>11.1</td>
<td>2.2</td>
<td>2</td>
<td>5.8</td>
</tr>
<tr>
<td>2. Hot nuclei + cytosol</td>
<td>+</td>
<td>38.1</td>
<td>5.4</td>
<td>5.7</td>
<td>2.4</td>
<td>16.6</td>
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<tr>
<td>3. Hot nuclei + buffer</td>
<td>+</td>
<td>92.8</td>
<td>7.5</td>
<td>7.6</td>
<td>5.7</td>
<td>10.2</td>
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<tr>
<td>4. Unlabeled nuclei + buffer</td>
<td>+</td>
<td>102.8</td>
<td>25.4</td>
<td>3.1</td>
<td>2.8</td>
<td>4.5</td>
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<tr>
<td>5. No nuclei + cytosol[3H]BP</td>
<td>+</td>
<td>86.5</td>
<td>25.4</td>
<td>4.5</td>
<td>2.7</td>
<td>40.4</td>
</tr>
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</table>

<sup>a</sup> Radioactivity eluting before BP, expressed as a fraction of the total eluting cpm.

<sup>b</sup> Results are the percentage of radioactivity cochromatographing with each marker compound normalized for the radioactivity emerging before BP. Products described may contain metabolites, other than the marker compounds, (36). Data shown are of a representative experiment.

<sup>c</sup> Fraction of total metabolites associated with the available marker compounds.
files and those published for rat liver nuclei (4–6, 10) and microsomes (17–18, 20, 32, 36) were present.

Nuclei readily take up BP from a solution in the absence of cytosol and may be expected to do so, perhaps less readily, in the intact cell. Since the BP exogenously added to nuclei appears to be metabolized no differently than translocated BP, a substantial role of metabolism in the induction process may not be indicated. After transport into nuclei, the ligand would dissociate from the hydrocarbon:protein complex, perhaps after interaction of the protein component with DNA. Further interaction with DNA or chromatin components may serve to regulate the expression of the cytochrome P-450 gene before final metabolism by nuclear mixed-function oxidase and epoxide hydrolase. The metabolism of polycyclic hydrocarbons prior to induction of cytochrome P-450c is not believed to be a prerequisite (2, 22). Our results are consistent with this idea. The nature of the association of BP with nuclear components and the results of this interaction are presently under study in our laboratory.

REFERENCES

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