

INDUCTION OF ARYL HYDROCARBON (BENZO[*a*]PYRENE) HYDROXYLASE AND TYROSINE AMINOTRANSFERASE IN HEPATOMA CELLS IN CULTURE

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ABSTRACT

In the Reuber (H35) hepatoma cell strain, microsomal aryl hydrocarbon (benzo[*a*]pyrene) hydroxylase is induced 25-fold by the polycyclic hydrocarbon benz[*a*]anthracene but is not induced by the steroid hormone dexamethasone. Soluble tyrosine aminotransferase is induced sixfold by dexamethasone and twofold by benz[*a*]anthracene. Each enzyme requires similar inducer concentrations for induction, and their induction kinetics are similar. The induction of each enzyme requires RNA and protein synthesis; in each case the transcriptional and translational steps can occur independently. The two induction systems are differentially sensitive to inhibitors of macromolecular synthesis. Simultaneous exposure to both inducers produces increases in both enzyme activities that are greater than those produced by either inducer alone. Each inducer acts at a pretranslational level to produce this synergistic effect. The results suggest that the requirements for macromolecular synthesis are similar for the induction of each enzyme, but that the turnover of enzyme-specific macromolecules may differ for each.

Aryl hydrocarbon (benzo[*a*]pyrene) hydroxylase is a substrate-inducible, microsomal mixed-function oxygenase found in most tissues of various species (1-4). This membrane-bound enzyme complex plays an important role in both the detoxification of carcinogenic polycyclic hydrocarbons and the conversion of some to more reactive forms (5-9). Tyrosine aminotransferase (EC 2.6.1.5) is a soluble enzyme inducible by steroid hormones both in rat liver *in vivo* and in hepatoma cells in culture (10-12). The mechanism of transaminase induction has been studied with inhibitors of macromolecular synthesis and by using antiserum to mea-

sure enzyme protein directly. Studies of hydroxylase induction in several cell culture systems, using inhibitors of macromolecular synthesis, suggest that hydroxylase activity is regulated through a mechanism similar to that proposed for the regulation of tyrosine aminotransferase (13-17). We have further examined this hypothesis by studying the simultaneous induction of both enzymes in the Reuber (H35) hepatoma cell strain. In these cells aryl hydrocarbon hydroxylase is inducible by the polycyclic hydrocarbon benz[*a*]anthracene (18), and tyrosine aminotransferase is inducible by the steroid hormone dexamethasone (11).

MATERIALS AND METHODS

Materials

Materials were obtained as follows: cell culture medium, serum, and antibiotics from Grand Island Biological Co., Grand Island, N.Y.; cell culture plates from Falcon Plastics Div. of B.-D. Laboratories, Los Angeles, Calif.; L-[4, 5-³H(N)]leucine (30.0–50.2 Ci/mmol) and [5-³H]uridine (25.7 Ci/mmol) from New England Nuclear, Boston, Mass.; NADPH, actinomycin D, and cycloheximide from Calbiochem, South Pasadena, Calif.; ribonuclease A and chromatographically purified collagenase (CLSFA) from Worthington Biochemical Corp., Freehold, N.J.; dexamethasone, α -ketoglutaric acid, pyridoxal phosphate, and 3'-deoxyadenosine from Sigma Chemical Corp., St. Louis, Mo.; L-tyrosine and 4-hydroxybenzaldehyde from Aldrich Chemical Co., Inc., Milwaukee, Wis.; trypsin (1-300) from Nutritional Biochemicals Corporation, Cleveland, Ohio; PCS solubilizer from Amersham/Searle Corp., Arlington Heights, Ill.; RPI scintillator from Research Products International Corp., Elk Grove Village, Ill.; benz[a]anthracene from K and K Laboratories, Inc., Plainview, N.Y.; benzo[a]pyrene from Eastman Kodak Co., Rochester, N.Y. Both polycyclic hydrocarbons were recrystallized from 95% ethanol and checked for purity by two-dimensional thin-layer chromatography using benzene-methanol (19:1) as solvents in the first direction and benzene-hexane (15:1) in the second direction.

Methods

CELL CULTURE: The Reuber (H35) hepatoma strain is a permanent cell line, derived from an acetylaminofluorene-induced minimal deviation hepatoma (19) and adapted to culture by Pitot et al. (20). The cells have been maintained in serial passage by Dr. Francis T. Kenney since 1964. Cells were grown in Ham's mixture F-12 (21) containing 5% fetal calf serum (vol/vol), 100 U/ml of penicillin, 100 μ g/ml of streptomycin, and 2.5 μ g/ml of amphotericin B, in a 5% CO₂ humidified atmosphere in a National incubator (National Instrument, Baltimore, Md.). Cells were maintained in serial passage using a solution of 0.1% trypsin (wt/vol), 2% chicken serum (vol/vol), and 12 units collagenase per ml of calcium- and magnesium-free Earle's balanced salt solution (22). For long-term storage, cells were suspended in F-12 medium containing 5% fetal calf serum plus 10% dimethylsulfoxide (vol/vol) and maintained at the temperature of liquid nitrogen. Experiments were performed on monolayers 5–10-days old in exponential growth. Fresh growth medium was added to the cultures 16 h before the start of each experiment.

PREPARATION OF INDUCING MEDIUM: Since fetal calf serum may contain hormones and/or other substances capable of affecting enzyme activity, experiments were performed in serum-free medium. Benz[a]anthracene, dissolved in dimethylsulfoxide, was added

directly to F12 medium. The final concentration of dimethylsulfoxide in the medium was 0.1%; this had no detectable effect on cell growth or enzyme induction. Dexamethasone was dissolved in a minimal amount of 100% ethanol, and F12 was added to make a 10⁻⁸ M stock solution. Aliquots of this stock solution were added directly to F12.

ENZYME ASSAYS: Cells were washed twice with chilled Dulbecco's phosphate-buffered saline, collected by scraping into 5 ml of chilled phosphate-buffered saline, and centrifuged at 1,000 g for 10 min at 0°C. The pellet was homogenized in 1.0 ml of 0.2 M potassium phosphate buffer, pH 7.60, and an aliquot assayed for hydroxylase activity by the method of Nebert and Gelboin (23). Incubation mixtures contained in a volume of 1.0 ml: 50 μ mol of Tris-HCl, pH 7.60; 3 μ mol of MgCl₂; 0.5 mg of NADPH; 200 μ l of cell homogenate; and 100 nmol of the substrate, benzo[a]pyrene, added in 40 μ l of methanol. Incubations were carried out for 30 min at 37°C and were performed under the illumination of a General Electric 25-W red bulb (General Electric Co., Richmond Heights, Ohio). The reaction was stopped by the addition of 1.0 ml of acetone, and the mixture was shaken with 3.0 ml of hexane for 10 min. A 1.0-ml aliquot of the acetone-hexane layer was extracted with 1.0 ml of 1 N NaOH, and the fluorescence of this extract was measured immediately at 396-nm excitation and 522-nm emission in an Aminco-Bowman spectrofluorometer (American Instrument Co., Inc., Travenol Laboratories, Inc., Silver Spring, Md.), and compared to the fluorescence of a standard 3-hydroxybenzo[a]pyrene solution. One unit of hydroxylase activity catalyzes in 1 min the formation of phenolic products with the fluorescence equivalent to 1 pmol of 3-hydroxybenzo[a]pyrene.

After the removal of aliquots for the measurement of hydroxylase activity and protein concentration, the remaining cell homogenate was centrifuged at 1,000 g for 5 min at 0°C. The supernate was used for the measurement of both tyrosine aminotransferase activity, according to the method of Diamondstone (24), and protein concentration. Incubation mixtures contained a volume of 3.1 ml: 2.8 ml of 0.2 M potassium phosphate buffer, pH 7.60, containing 19.2 μ mol of L-tyrosine, 0.1 ml of a buffer solution of 0.3 M α -ketoglutarate, 0.1 ml of a buffer solution of 1.2 mM pyridoxal phosphate, and 0.1 ml of the supernate. Incubations were carried out for 30 min at 37°C. The reaction was stopped by the addition of 0.2 ml of 10 N NaOH with immediate shaking. After 30 min at room temperature the absorbancy at 331 nm was read in a Gilford spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio). A zero-time control was prepared for each measurement by adding NaOH to the incubation mixture before addition of the α -ketoglutarate. One unit of transaminase activity catalyzes in 1 min the production of 1 nmol of 4-hydroxybenzaldehyde.

Both enzyme reactions were linear with respect to time

of incubation and amount of cellular protein. Protein concentration was determined by the method of Lowry et al. (25) with ribonuclease A as standard.

MEASUREMENTS OF RNA AND PROTEIN SYNTHESIS: RNA specific activity was measured by the method of Fleck and Munro (26). The RNA was assayed for radioactivity in 10 ml PCS in a Packard scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.), and for absorbance at 260 nm in a Gilford spectrophotometer. Proteins were purified by the method of Siekevitz (27). The trichloroacetic acid-precipitable residue was hydrolyzed at 60°C for 20 min in 1 N NaOH and aliquots were assayed for radioactivity in 10 ml PCS and for protein by the method of Lowry et al. (25).

RESULTS

Kinetics of Enzyme Induction

Fig. 1 shows that during exposure to benz[*a*]anthracene (1 $\mu\text{g}/\text{ml}$), hydroxylase activity increases 25-fold over the first 8 h; this is followed by a gradual decline in enzyme activity over the next 16 h. There is no increase in hydroxylase activity upon exposure to control medium. Tyrosine aminotransferase activity increases three- to four-fold during the first 4 h of exposure to benz[*a*]anthracene and then decreases to control levels; control medium alone also produces a twofold stimulation of tyrosine aminotransferase activity. Transaminase activity remains elevated if benz[*a*]anthracene is readded to the cultures after the initial 4-h exposure; in addition, the transaminase is induced to similar levels by the polycyclic hydrocarbons 3-methylcholanthrene, dibenz[*a,h*]anthracene, and 9,10-dimethylbenz[*a*]anthracene (data not shown). Thus, exposure of the cells to benz[*a*]anthracene (or other polycyclic hydrocarbons) increases the activity of both enzymes but the increase in hydroxylase activity is much greater and of longer duration than the increase in transaminase activity.

Fig. 2 shows that tyrosine aminotransferase activity increases about sixfold during the first 8 h of exposure to dexamethasone and remains at this increased level during the next 16 h; again, there is a small increase in transaminase activity 4 h after exposure to control medium. Dexamethasone has no stimulatory effect on hydroxylase activity. Thus, while benz[*a*]anthracene increases the activity of both enzymes, dexamethasone only stimulates transaminase activity. The reason(s) for this difference is unknown, however, it indicates that the two induction mechanisms differ in their responses to these inducers.

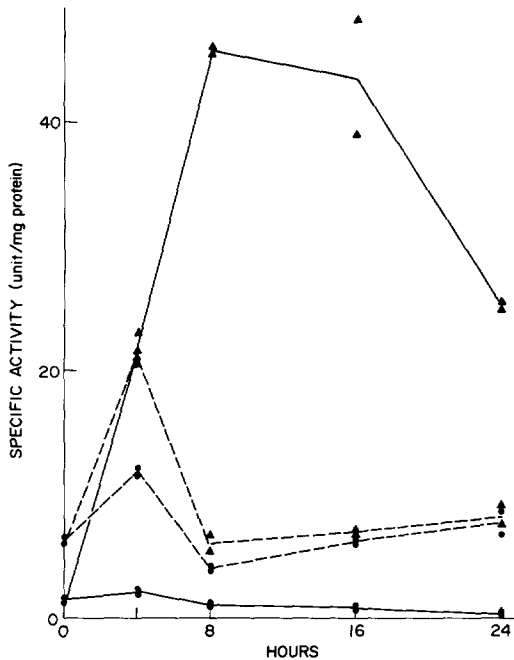


FIGURE 1 Effect of benz[*a*]anthracene on aryl hydrocarbon hydroxylase activity (solid lines) and tyrosine aminotransferase activity (dashed lines). Cells were exposed for the times indicated to either F12 medium alone (●) or F12 medium containing benz[*a*]anthracene, 1 $\mu\text{g}/\text{ml}$ (▲). In this and in all subsequent figures, each point represents the results from one plate of cells. A single measurement of tyrosine aminotransferase activity and duplicate measurements of both aryl hydrocarbon hydroxylase activity and protein concentration were made in determining enzyme specific activity.

Upon exposure to its respective inducer, each enzyme increases rapidly in activity for about 8 h. During the next 16 h, hydroxylase activity declines and transaminase activity remains elevated. This difference in behavior is probably due to the metabolism of benz[*a*]anthracene by the hydroxylase, with the consequential decrease in inducer concentration. Dexamethasone, on the other hand, is not metabolized by the transaminase, and the effective concentration of this inducer is probably maintained over the full period of observation. Fig. 3 indicates that the lag period preceding increased enzyme activity is about 1 h for both enzymes, suggesting that the biochemical events necessary for hydroxylase induction and transaminase induction require similar periods of time.

Effect of Low Inducer Concentrations

Fig. 4 shows that a 4-h exposure to concentrations of benz[*a*]anthracene of 10^{-8} M or greater

produces substantial hydroxylase induction and that dexamethasone induces tyrosine aminotransferase at similar concentrations. The results suggest that the sensitivity of each induction mechanism to its respective inducer is similar.

The results also indicate that dexamethasone produces no increase in hydroxylase activity at any of the concentrations tested. In contrast, benz[*a*]anthracene does induce tyrosine aminotransferase, although the degree of induction is less than produced by an equimolar concentration of dexamethasone. This again indicates that the two induction mechanisms differ in their responses to these inducers.

Effect on Enzyme Induction of Inhibitors of Macromolecular Synthesis

Actinomycin D, 3'-deoxyadenosine (cordycepin), and cycloheximide each inhibits the induction of both enzymes, suggesting that both induction processes require RNA and protein synthesis. None of these inhibitors produces morphological evidence of toxicity.

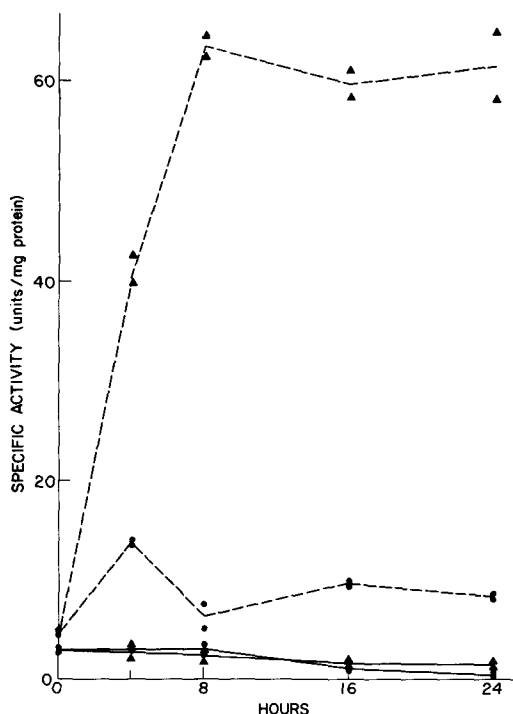


FIGURE 2 Effect of dexamethasone on aryl hydrocarbon hydroxylase activity (solid lines) and tyrosine aminotransferase activity (dashed lines). Cells were exposed for the times indicated to either F12 medium alone (●) or F12 medium containing dexamethasone, 1 μ M (▲).

Fig. 5 A and 5 B indicate that actinomycin D (1 μ g/ml) and 3'-deoxyadenosine (25 μ g/ml), at concentrations which reduce total RNA synthesis by about 85% and 50%, respectively, completely inhibit both hydroxylase induction and transaminase induction. The results suggest that RNA synthesis is required for the induction of both enzymes. 3'-Deoxyadenosine has been shown to inhibit preferentially the synthesis of polyadenylic acid sequences, an event presumed necessary in the biogenesis of certain messenger RNAs (28). Thus, our results are consistent with the idea that messenger RNA is the species whose synthesis is required for the induction of each enzyme.

Fig. 6 shows that the induction of both enzymes is completely prevented by cycloheximide (1 μ g/ml) at a concentration which inhibits protein synthesis by 92%. These results indicate that the induction of each enzyme requires protein synthesis. Fig. 5 A and 5 B also indicate that low concentrations of either actinomycin D or 3'-deoxyadenosine inhibit transaminase induction to a somewhat greater extent than hydroxylase induction. In contrast, Fig. 6 shows that low concentrations of cycloheximide inhibit hydroxylase induction more than transaminase induction. These differential effects may indicate that the turnover of enzyme-specific RNA and/or protein is different for each enzyme.

The relationship between transcription and translation during enzyme induction is shown in Fig. 7. When cells are exposed for 4 h to both inducers in the presence of cycloheximide (1 μ g/ml), there is no increase in enzyme activity. If the inhibitor is then removed, both enzyme activities rise; these increases occur in the presence of actinomycin D (1 μ g/ml) or 3'-deoxyadenosine (25 μ g/ml) at concentrations which completely prevent enzyme induction under other conditions (Fig. 5). The rise in enzyme activity requires protein synthesis, since readdition of cycloheximide inhibits the increase. These results indicate that the processes of transcription and translation can occur independently for the induction of both enzymes. Presumably, during the period of cycloheximide treatment, both hydroxylase-specific RNA and transaminase-specific RNA are synthesized, but not translated. After the block in protein synthesis has been removed, these RNAs can be translated in the absence of further transcription.

Fig. 7 also indicates that after removal of the block in protein synthesis, hydroxylase activity continues to rise for at least 4 h in the presence of

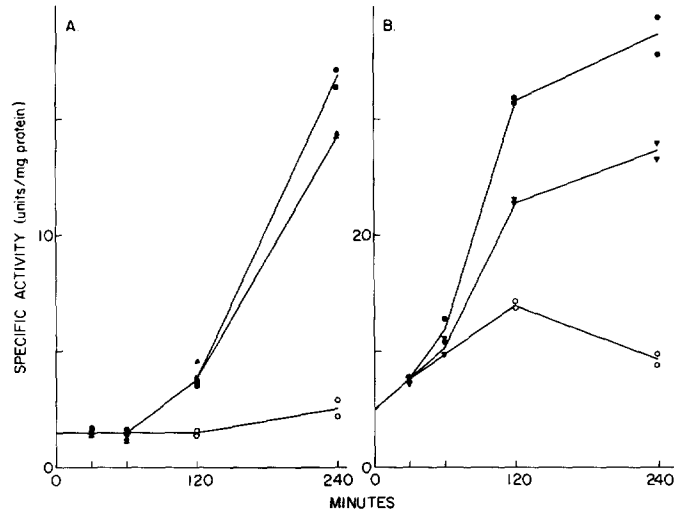


FIGURE 3 Early kinetics of induction of (A) aryl hydrocarbon hydroxylase and (B) tyrosine aminotransferase. Cells were exposed for the times indicated to F12 medium alone (O—O) or to F12 medium containing benz[a]anthracene, 1 $\mu\text{g}/\text{ml}$ (▲—▲), dexamethasone, 1 μM (▼—▼) or benz[a]anthracene plus dexamethasone (●—●).

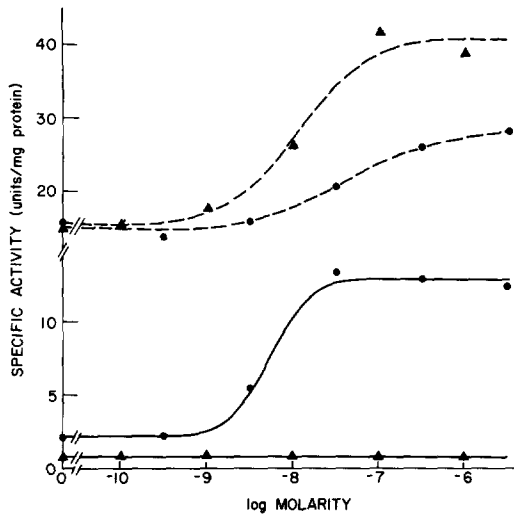


FIGURE 4 Effect of the concentration of benz[a]anthracene or dexamethasone on the activity of aryl hydrocarbon hydroxylase (solid lines) and tyrosine aminotransferase (dashed lines). Cells were exposed for 4 h to F12 medium containing either benz[a]anthracene (●) or dexamethasone (▲) at the indicated concentrations. A single measurement of tyrosine aminotransferase activity and duplicate measurements of both aryl hydrocarbon hydroxylase activity and protein concentration were made for each determination of enzyme specific activity. Each point represents the average of measurements from two plates of cells. The variation in specific activities from identically-treated plates of cells was less than 10%.

actinomycin D (or 3'-deoxyadenosine), while transaminase activity increases for only 2 h under identical conditions. There are at least two possible explanations for this phenomenon. Less transaminase-specific RNA than hydroxylase-specific RNA may accumulate due to its more rapid turnover. This would lead to an earlier cessation of transaminase synthesis. A second possibility is that the turnover of transaminase protein may be increased relative to that of the hydroxylase, resulting in a lesser increase in transaminase activity.

Simultaneous Induction of Both Enzymes

Fig. 8 shows the effects on enzyme induction of the simultaneous administration of both benz[a]anthracene and dexamethasone, compared to the effects of each inducer administered separately. Dexamethasone induces the transaminase but not the hydroxylase, and benz[a]anthracene markedly increases hydroxylase activity and increases transaminase activity slightly. In the presence of both inducers, however, both enzyme activities increase to levels higher than those produced by either inducer alone. This synergistic effect is particularly pronounced for hydroxylase activity; there is a similar, but less marked effect on transaminase activity.

The results in Table I suggest that this synergis-

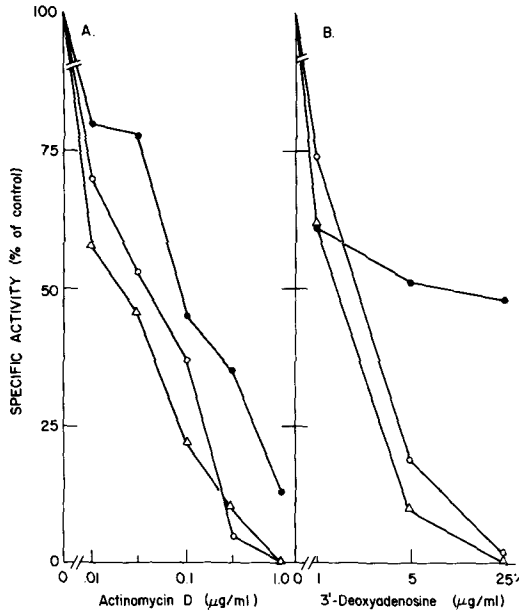


FIGURE 5 Effect of (A) actinomycin D or (B) 3'-deoxyadenosine on aryl hydrocarbon hydroxylase induction, tyrosine aminotransferase induction, and RNA synthesis. Cells were exposed for 4 h to F12 medium containing benz[*a*]anthracene (1 μg/ml), dexamethasone (1 μM), and the indicated concentration of (A) actinomycin D or (B) 3'-deoxyadenosine. Hydroxylase activity (O—O) and transaminase activity (Δ—Δ) are expressed as the percent of enzyme induction in the absence of inhibitor. Other plates were exposed for 1 h to medium containing both inducers plus the indicated concentration of actinomycin D or 3'-deoxyadenosine. [5-³H]uridine (1 μCi/ml) was then added for 1 h and the specific activity of RNA was determined (●—●). The degree of inhibition of RNA synthesis remains at the indicated level for at least 4 h (data not shown). Each point represents the average of determinations from duplicate plates of cells.

tic effect is due to action(s) of these inducers on RNA metabolism. Thus, dexamethasone, when administered with benz[*a*]anthracene, produces an increase in hydroxylase activity only under conditions which allow RNA synthesis. Similarly, benz[*a*]anthracene, when administered with dexamethasone, produces a rise in transaminase activity only under conditions which allow transcription. Whether these inducers act by stimulating transcription per se, or by stabilizing a specific cytoplasmic mRNA or nuclear precursor(s) is unknown; the net effect appears to be the accumulation of an induction-specific RNA. These results suggest that, in addition to their activities as inducers of specific enzymes, both benz[*a*]an-

thracene and dexamethasone have other biological similarities with respect to their effects on RNA metabolism.

DISCUSSION

In vivo, hepatic aryl hydrocarbon hydroxylase activity increases not only after exposure to polycyclic hydrocarbons, but also after exposure to drugs, pesticides, and other exogenous compounds. Furthermore, adrenalectomy, hypophysectomy, or administration of certain steroid hormones may

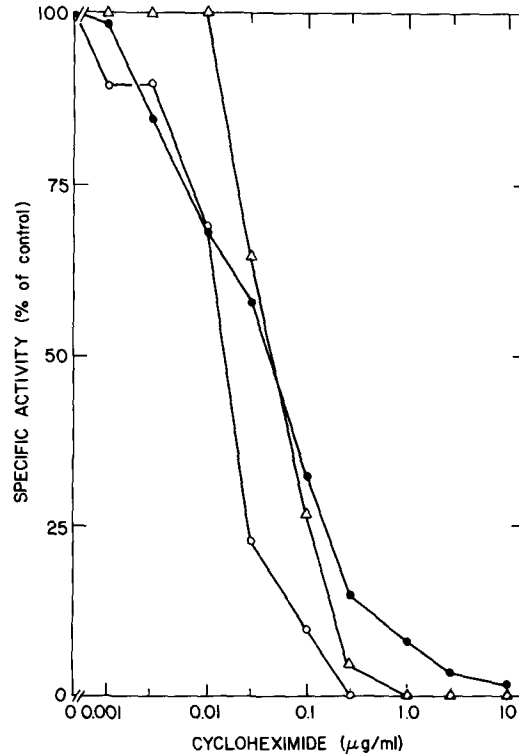


FIGURE 6 Effect of cycloheximide on aryl hydrocarbon hydroxylase induction, tyrosine aminotransferase induction, and protein synthesis. Cells were exposed for 4 h to F12 medium containing benz[*a*]anthracene (1 μg/ml), dexamethasone (1 μM), and the indicated concentration of cycloheximide. Hydroxylase activity (O—O) and transaminase activity (Δ—Δ) are expressed as the percent of enzyme induction in the absence of inhibitor. Other plates were exposed for 1 h to medium containing both inducers plus the indicated concentration of cycloheximide. L-[4,5-³H]leucine (1 μCi/ml) was then added for 1 h and the specific activity of cellular proteins was determined (●—●). The degree of inhibition of protein synthesis remains at the indicated level for at least 4 h (data not shown). Each point represents the average of determinations from duplicate plates of cells.

alter the activity of hepatic drug-metabolizing enzyme systems; such findings suggest that the hormones of the pituitary-adrenal axis are involved in the regulation of drug-metabolizing enzymes. In addition, the hepatic enzymes which hydroxylate steroid hormones have properties similar to those of drug-metabolizing enzyme systems (29, 30). Thus, there is a complex interrelationship between steroid hormones, polycyclic hydrocarbons, and microsomal hydroxylases.

An understanding of the regulation of aryl hydrocarbon hydroxylase is relevant to several problems. First, the enzyme complex plays an important role in the metabolism of carcinogenic polycyclic hydrocarbons (5-9), and thus its activity may be an important factor in chemical carcinogenesis. Second, the hydroxylase is typical of the microsomal drug-metabolizing enzyme complexes involved in the detoxification of many exogenous compounds, including carcinogens, pesticides, and drugs (29-31); an understanding of the regulation of such membrane-associated en-

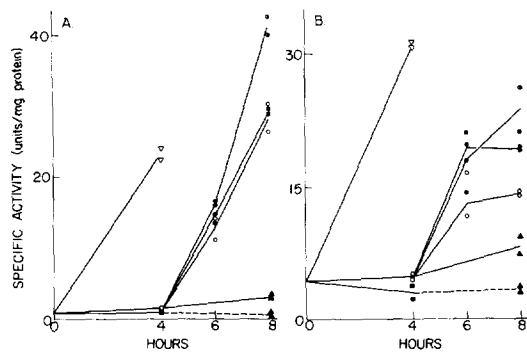


FIGURE 7 Independence of transcription and translation in (A) aryl hydrocarbon hydroxylase induction and (B) tyrosine aminotransferase induction. Cells were exposed for 4 h to F12 medium containing benz[*a*]anthracene and dexamethasone (∇), or benz[*a*]anthracene and dexamethasone plus cycloheximide (\square). Following two 15-min washes with the second medium, they were exposed to F12 medium containing both inducers alone (\bullet — \bullet), both inducers plus actinomycin D (\circ — \circ), or both inducers plus actinomycin D and cycloheximide (\blacktriangle — \blacktriangle). Other plates were exposed for 4 h to F12 medium containing benz[*a*]anthracene and dexamethasone plus 3'-deoxyadenosine (\blacksquare). After two 15-min washes with the second medium, they were exposed to F12 medium containing both inducers plus actinomycin D (\blacktriangle — \blacktriangle). Concentrations used were: benz[*a*]anthracene, 1 μ g/ml; dexamethasone, 1 μ M; actinomycin D, 1 μ g/ml; cycloheximide, 1 μ g/ml; 3'-deoxyadenosine, 25 μ g/ml.

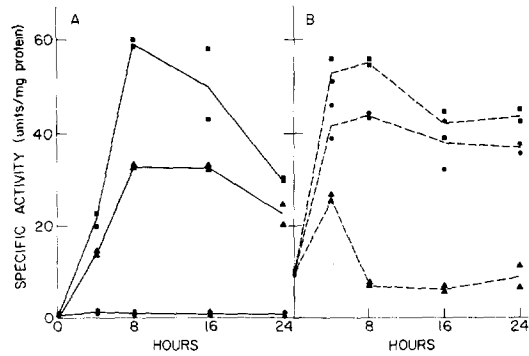


FIGURE 8. Combined effect of benz[*a*]anthracene and dexamethasone on (A) aryl hydrocarbon hydroxylase activity and (B) tyrosine aminotransferase activity. Cells were exposed for the times indicated to F12 medium containing benz[*a*]anthracene, 1 μ g/ml (\blacktriangle), dexamethasone, 1 μ M (\bullet), or benz[*a*]anthracene plus dexamethasone (\blacksquare).

zymes may lead to a better understanding of the effects of environmental pollutants and other xenobiotics on the cell. Third, the hydroxylase system is both inducible and part of a multienzyme membrane complex; knowledge of the biochemical events necessary for induction may contribute to the understanding of the regulation of other inducible membrane-bound mammalian enzymes.

Tyrosine aminotransferase is an inducible mammalian enzyme whose regulation has been extensively studied (10-12). These investigations have been facilitated by the ability to purify the transaminase and prepare specific antiserum. Studies in hepatoma tissue culture (HTC) cells indicate that the increase in enzyme activity which follows exposure to dexamethasone is accompanied by a corresponding increase in antigenic material precipitable with antitransaminase antiserum. Such experiments indicate that the steroid-induced increases in enzymatic and antigenic activities can be accounted for on the basis of an increased rate of enzyme synthesis (11, 32).

Analogous studies of hydroxylase induction, using antihydroxylase antiserum, have not been possible; the microsomal nature of the enzyme complex has made its purification difficult. However, studies using inhibitors of macromolecular synthesis have suggested that hydroxylase induction involves biochemical events similar to those required for transaminase induction. Our studies indicate that the requirements for macromolecular synthesis are similar for the induction of both the

TABLE I
Effect of Inhibitors of Macromolecular Synthesis on the Simultaneous Induction of Aryl Hydrocarbon Hydroxylase and Tyrosine Aminotransferase*

	Treatments†		Hydroxylase activity§	Transaminase activity§
	Initial	Secondary		
Control medium		None	3.6	8.5
Benz[a]anthracene		None	12.9	—
Dexamethasone		None	—	17.2
Benz[a]anthracene + dexamethasone		None	23.6	34.0
Benz[a]anthracene + cycloheximide		None	1.4	—
Dexamethasone + cycloheximide		None	—	5.8
Benz[a]anthracene + dexamethasone + cycloheximide		None	1.2	81
Benz[a]anthracene + cycloheximide		Benz[a]anthracene + actinomycin D	18.5	—
Benz[a]anthracene + cycloheximide		Benz[a]anthracene + dexamethasone + actinomycin D	17.2	—
Benz[a]anthracene + dexamethasone + cycloheximide		Benz[a]anthracene + actinomycin D	27.3	—
Benz[a]anthracene + dexamethasone + cycloheximide		Benz[a]anthracene + dexamethasone + actinomycin D	26.8	—
Dexamethasone + cycloheximide		Dexamethasone + actinomycin D	—	16.9
Dexamethasone + cycloheximide		Dexamethasone + benz[a]anthracene + actinomycin D	—	14.9
Dexamethasone + benz[a]anthracene + cycloheximide		Dexamethasone + actinomycin D	—	33.6
Dexamethasone + benz[a]anthracene + cycloheximide		Dexamethasone + benz[a]anthracene + actinomycin D	—	27.6

* Values are the averages from duplicate plates of cells. Results from identically treated plates of cells varied less than 10%. (—) indicates that no measurement was made. Concentrations used: see legend for Fig. 7.

† Treatments were for 4 h, except as noted.

§ Units per milligram of protein.

|| 2-h treatments.

membrane-bound hydroxylase and the soluble transaminase. Both induction mechanisms involve (presumably messenger) RNA synthesis and protein synthesis, and these events can occur independently and may be separated experimentally (10, 15).

The half-time of degradation of tyrosine aminotransferase activity is 3–7 h, depending on the experimental conditions (11, 12). In analogous experiments, the half-time of degradation of hydroxylase activity is 3–10 h, depending on the conditions and the cell type (13, 33, 34). The half-lives of the presumed enzyme-specific RNAs are unknown. Our results indicate that in this cell strain, low concentrations of inhibitors of macromolecular synthesis differentially inhibit the induction of these two enzymes, suggesting that the turnover rates of enzyme-specific RNA and/or protein are different for the two enzymes.

Various steroids have different activities with respect to the induction of tyrosine aminotransferase. On the basis of studies in HTC cells, steroids have been grouped into four classes: (a) optimal inducers, such as dexamethasone, induce the enzyme to "maximal" levels; (b) suboptimal inducers induce the enzyme to lower levels and competitively inhibit induction by optimal inducers; (c) anti-inducers do not induce the enzyme and inhibit induction by optimal and suboptimal inducers; (d) inactive steroids neither induce nor inhibit induction. These results have been interpreted in terms of an allosteric receptor system involved in the control of enzyme induction (35). Benz[a]anthracene does not fit into any of these inducer classes. Benz[a]anthracene alone induces tyrosine aminotransferase to "suboptimal" levels; however, when coadministered with the optimal inducer, dexamethasone, it potentiates rather

than inhibits transaminase induction. This synergistic effect may indicate that benz[a]anthracene acts through a mechanism different from dexamethasone to stimulate the induction of tyrosine aminotransferase.

There are several conceivable mechanisms whereby the simultaneous induction of two apparently unrelated enzymes might result in higher activities for both than the induction of either enzyme alone. (a) The general process of enzyme induction may have a rate-limiting step involving the synthesis or activation of a component(s) of the protein-synthesizing apparatus. The simultaneous induction of two enzymes might increase the availability of such a rate-limiting component, allowing increased induction of both enzymes. (b) In addition to their presumed action of promoting the accumulation of induction-specific (messenger) RNA, both inducers may have the general property of promoting the overall accumulation of translatable RNA, either by stimulating the transcription of the genome, or by stabilizing cytoplasmic mRNA and/or its nuclear precursor(s). (c) The genome for each enzyme may contain inter-related control elements or a "cascading" regulatory circuit, as suggested by Pontecorvo (36). In any event, our findings are relevant to the understanding of the biological effects of steroid hormones and to the effect on the cell of polycyclic hydrocarbons, which are important environmental pollutants (37).

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