Escherichia coli, all five enzymes of the pathway are repressed by uracil, but the repression is coordinate only in the case of the last four (*10*). In yeast, the first enzyme of the pathway is repressed by uracil, while the second is induced by its own substrate, and the final three are believed to be induced by dihydroorotic acid (*15*). Our data suggest that, for cultured human cells, the final two enzymes are affected by a Mendelian mutation and respond concurrently to several inhibitors of the UMP pathway. Neither the gene nor the inhibitors significantly affect the activity of DAD. Hence in the human cell the last two enzymes of the pathway may respond separately from the enzyme that immediately precedes them.

However, we must emphasize that: (i) Because of imprecision in the assays we have not shown that OMP decarboxylase and OMP pyrophosphorylase are exactly coordinate. (ii) The molecular mode of action of the gene for orotic aciduria is not known. (iii) We have not shown that the increase in OMP pyrophosphorylase and OMP decarboxylase activity, in response to the inhibitors, is due to enzyme induction. We do not know whether it reflects a preferential stimulation enzyme synthesis, or, say, a decelerated rate of enzyme destruction. In cultured human cells these two possibilities are particularly difficult to distinguish (*16*).

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14. In this experiment barbituric acid was used at a concentration of 1.2 millimolar, whereas in the experiment showing inhibition in cell-free extracts the concentration was 25 millimolar. Barbituric acid could not feasibly be applied to whole cells at the higher concentration, because even 10 m*M* barbituric acid will prevent the growth of diploid cell strains, whether cytidine is present or not. The high concentration of barbituric acid used in the cell-free experiments was made necessary by the large excess of substrate present in the reaction mixture. Since the inhibition is competitive, a 50-percent decrease in velocity can theoretically be obtained over a wide range of concentrations of barbituric acid by making an appropriate change in substrate concentration. However, because of the limited sensitivity of the spectrophotometric assay, it is necessary to use high concentrations of substrate (to obtain maximum reaction velocity and thus ensure that catalytic activity can be accurately measured). The concentration of substrate in the cell is almost certain to be much less than the concentration ($10^{-3}M$) used in the reaction mixture.
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Cigarette Smoking: Stimulatory Effect on Metabolism of 3,4-Benzpyrene by Enzymes in Human Placenta

Abstract. *The enzymatic hydroxylation of 3,4-benzpyrene was not detected in human placentas obtained after childbirth from nonsmokers, whereas this enzyme activity was present in placentas obtained from individuals who smoked cigarettes. The degree of induction of benzpyrene hydroxylase caused by cigarette smoking varied in different individuals. Treatment of pregnant rats with benzpyrene increased the activity of this hydroxylase in the placenta.*

3,4-Benzpyrene (BP) and several other polycyclic aromatic hydrocarbons are environmental carcinogens that are present in polluted city air (*1*), certain smoked or cooked foods (*2*) and tobacco smoke (*3*). Studies on the metabolism of BP revealed in the liver microsomes of rats and humans an enzyme system dependent on the reduced form of nicotinamide-adenine dinucleotide phosphate (NADPH) that hydroxylates this carcinogen (*4, 5*). Because the hydroxylated metabolites of BP are less carcinogenic than the parent molecule, the enzymatic hydroxylation of BP is a detoxification mechanism.

The administration of BP or other polycyclic hydrocarbons to rats induces severalfold increases in BP-hydroxylase activity in the liver, lung, gastrointestinal tract, skin, and other tissues (*4, 6*). This adaptive response in rats probably provides protection from the carcinogenic effects of polycyclic aromatic hydrocarbons and other carcinogens. Indeed, treatment of rats with suitable enzyme inducers markedly inhibits the carcinogenic activities of such compounds as BP (*7*), 3′-methyl-4-dimethylaminoazobenzene (*8*), 2-acetylaminofluorene (*8*), and 9,10-dimethyl-1,2-benzanthracene (*9*). Hence, we wanted to evaluate the quantity of carcinogen-metabolizing enzymes in tissues of man and to determine if constant exposure of people to polycyclic hydrocarbons increases the quantity of enzymes that detoxify these compounds. Because BP and other polycyclic hydrocarbons are constituents of cigarette smoke, we now report the effect of cigarette smoking on BP-hydroxylase activity in human placenta —a readily obtainable human tissue.

Placentas were placed in a freezer at −15°C immediately after normal childbirth and were assayed for BP-hydroxylase activity within 48 hours. A pie-shaped section (6 to 10 g) of the placenta was homogenized in 0.25*M* sucrose solution to yield a 10-percent suspension. Homogenates of rat placenta were prepared in the same way, except that all placentas from each rat were pooled and the tissues were assayed immediately after the animal was killed. The homogenate (1 ml) was incubated aerobically with 50 μg of BP in the presence of an NADPH-generating system; the amount of 8-hydroxy-3,4-benzpyrene formed in 15 minutes was measured by the method of Kuntzman *et al.* (*5*). In that the activation and fluorescence spectra of BP metabolites formed by placenta were identical with those of authentic 8-hydroxy-3,4-benzpyrene in sodium hydroxide solution, the hydroxylation of BP was expressed as nanograms of 8-hydroxy-3,4-benzpyrene formed, even though the fluorescence measured may represent a mixture of hydroxylated metabolites. The amount of 8-hydroxy-3,4-benzpyrene formed by human or rat

Table 1. Effect of cigarette smoking on BP-hydroxylase activity in human placentas. For anesthesia during delivery, all patients received intermittent epidural injections of mepivacaine. One subject in the group of nonsmokers received 60 mg of phenobarbital daily for 8 months for epilepsy, one in the group of smokers received 100 mg of diphenylhydantoin daily for 3 months for psychomotor seizures, and another in the group of smokers received 45 mg of phenobarbital daily for 4 months for the treatment of hypertension.

Cigarettes smoked daily during pregnancy	Patients (No.)	Ages	8-Hydroxy-BP formed by placenta (ng/g per 15 min)
0	12	20–40 Av. 26	< 20
10–30 Av. 19	11	20–31 Av. 24	69–3680 Av. 756

placenta was linear with time and tissue concentration during at least 15 minutes. If linearity with tissue concentration was not obtained, the placenta was considered devoid of BP-hydroxylase activity. Little variation in this activity was found in different samples of the same placenta.

Placentas from 23 human subjects were assayed for BP-hydroxylase activity (Table 1). Only the placentas from the 11 women who smoked cigarettes showed appreciable BP-hydroxylase activity. The number of cigarettes smoked daily by the women varied from 10 to 30, but the BP-hydroxylase activity present in the placentas did not vary accordingly. Thus, the placenta from one patient who smoked 20 to 30 cigarettes daily had 14 to 16 times the BP-hydroxylase activity of those from two other patients who smoked similar numbers of cigarettes. A placenta from another patient who smoked 15 to 20 cigarettes daily had 2 to 3 times the BP-hydroxylase activity of those from two patients who smoked as many as 30 cigarettes daily. The lack of an apparent dose-response curve for the smokers may be explained by variation (i) in the amount of BP and other polycyclic hydrocarbons present in the smoke from different brands of cigarettes, (ii) in the manner of smoking a cigarette, or (iii) in the responses of various individuals to the enzyme inducers in cigarette smoke. No correlation was found between BP-hydroxylase activity in the placenta and race, age, or medication of the patient prior to delivery.

Table 2. Effects of various doses of BP on BP-hydroxylase activity (mean ± S.D.) in rat placenta. Thirty rats, pregnant for 19 days and weighing 225 to 250 g, were divided into ten groups. Nine of the groups received an oral dose of BP dissolved in corn oil, but the control group received only corn oil. The rats were killed 24 hours later, and the placentas were examined for BP-hydroxylase activity (8-hydroxy-3,4-benzpyrene formed per 15 minutes).

BP dose (mg/kg)	BP-hydroxylase activity (ng/g)
Control	< 40
0.001 and 0.01	< 40
.1	46 ± 14
.25	410 ± 75
.50	662 ± 135
1	839 ± 64
10	1098 ± 96
50	1672 ± 316
100	1974 ± 24

Because cigarette smoke contains BP as well as other polycyclic hydrocarbons, we investigated the effect of BP administration in vivo on BP-hydroxylase activity in rat placenta. Rats that had been pregnant for 19 days were given various doses of BP orally and were killed 24 hours later. The BP-hydroxylase activity in rat placenta was proportional to the amount of hydrocarbon administered (Table 2). The administration of 0.001 or 0.01 mg of BP per kilogram failed to induce the activity, whereas doses greater than 0.1 mg/kg brought about this activity, which increased with dosage.

Enzyme induction in animals after the administration of certain carcinogens and drugs has been recognized to alter markedly the actions of drugs (*10, 11*). Similarly, treatment of humans with several substances also enhances drug metabolism in vivo and alters drug actions in man (*11*), but no human enzyme data have been presented. The suggestion that smoking enhances the metabolism in vivo of nicotine in man may explain the tolerance to nicotine that occurs in human smokers (*12*).

We show, for the first time, that compounds present in cigarette smoke can induce an enzyme in human tissue capable of metabolizing the carcinogen, BP. The induction of carcinogen-metabolizing enzymes, such as BP-hydroxylase, may be a mechanism whereby people protect themselves and their fetuses from environmental carcinogens. Because the products of BP metabolism are less carcinogenic than the parent molecule (*13*), persons with large quantities of BP-hydroxylase in the lung and gastrointestinal tract may be less susceptible, and those with small quantities more susceptible, to the carcinogenic actions of polycyclic hydrocarbons ingested in various smoked or cooked foods or inhaled in cigarette smoke or polluted city air. Similarly, fetuses with placentas that have much BP-hydroxylase activity presumably would be less susceptible to environmental carcinogens than fetuses with placentas that have little hydroxylase activity would be. Possibly, genetic differences in the inducibility of enzymes that detoxify polycyclic hydrocarbons in the lung, gastrointestinal tract, skin, and placenta may play an important role in the susceptibilities of different individuals to the carcinogenic effect of polycyclic hydrocarbons in our environment. If this were true, the determination of BP-hydroxylase activity in the tissues of smokers and others exposed to polycyclic hydrocarbons would be of value in estimating the relative hazards faced by people exposed to these carcinogens.

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