# Induction of Benzo[$\alpha$]pyrene Hydroxylase in Human Skin

Abstract. *Foreskins from children who were circumcised 2 to 4 days after birth contain an enzyme system that hydroxylates the carcinogen benzo[α]pyrene. When foreskin was cultured for 16 hours in the presence of 10 micromolar benz[α]anthracene, a two- to fivefold increase in activity of benzo[α]pyrene hydroxylase was obtained. An evaluation of the basal activity and inducibility of carcinogen-metabolizing enzymes in human tissues may provide a means of determining the ability of different individuals to metabolize carcinogens.*

Treatment of rats with the carcinogen benzo[α]pyrene (BP), or certain other polycyclic hydrocarbons, enhances the hydroxylation of BP and various drugs by enzymes in liver and certain other tissues (*1, 2*). In addition, BP hydroxylase is induced in man; cigarette smoke contains BP and other polycyclic hydrocarbons, and pregnant women who smoke cigarettes have elevated concentrations of BP hydroxylase in their placentas (*3*). The degree of activity of this enzyme in human placenta varies widely among women smokers; this variation is apparently not dependent on the number of cigarettes smoked during pregnancy, which suggests the possibility of genetic differences in the inducibility of BP hydroxylase in man. Individual differences in the ability of humans to metabolize the carcinogen *N*-2-fluorenylacetamide (*4*) and several drugs (*5*) in vivo have also been demonstrated. Since humans are exposed to many chemical carcinogens in the environment (*6*), the question of whether variability in the induction of carcinogen metabolism can influence the development of certain human cancers should be considered.

The cell culture system is suited for studies on microsomal enzyme induction in mammalian tissues because such investigations in the intact animal are subject to many variables, including age, sex and species differences, hormonal and nutritional variations, and exposure to a variety of environmental chemicals. In addition, with the cell culture technique, the time of exposure and the concentration of inducer can be rigorously controlled. Recent studies have demonstrated the inducibility of BP hydroxylase in a cell culture system in which tissue from the hamster embryo is used (*7, 8*). In our study we examined the inducibility of BP hydroxylase in cultures of human foreskin. The possible use of this system to study variations in the occurrence and inducibility of micrsomal enzymes that metabolize chemical carcinogens in man is thus raised.

To 95 ml of Eagle's minimal essential medium containing Earle's balanced salt solution (Grand Island Biological) we added 5 ml of fetal bovine serum (Microbiological Associates), 1 ml of 0.2*M* glutamine, 10 mg of streptomycin, 2500 units mycostatin, and 10,000 units of penicillin G. Plastic tissue-culture dishes were obtained from Falcon Plastics. Medium containing 10 $\mu M$ benz[α]anthracene (BA) was prepared according to the method of Nebert and Gelboin (*7*).

Human foreskins were obtained from children circumcised 2 to 4 days after birth and were immediately placed in control medium. For the surgery, the skin was lightly swabbed with 70 percent isopropyl alcohol, and a Yellen-Gomco clamp was used. After the removal of fatty tissue, each skin was weighed and divided into two pieces: one piece was placed in control medium, and the other piece in the medium containing 10 $\mu M$ BA. The skins were minced in the medium and subsequentially maintained at 37°C for 16 hours in a humidified atmosphere of 5 percent $CO_2$ in air. The medium containing the minced skin was then centrifuged at 10,000*g* for 1 minute, and the pellet was washed twice with 5 ml of 0.1*M* $K_2HPO_4$-$KH_2PO_4$ buffer (*p*H 7.4). The skin pellet was blotted on filter paper and immediately immersed in liquid nitrogen.

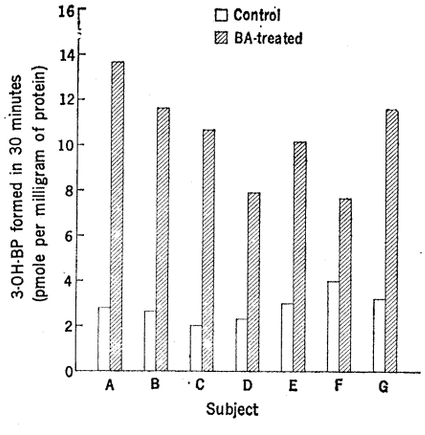


Fig. 1. Induction of BP hydroxylase in foreskin tissue culture. Minced foreskin was incubated for 16 hours in culture medium, in the presence or absence of 10 $\mu M$ benz[α]anthracene (BA).

Foreskins stored in liquid nitrogen were removed and immediately ground to a fine powder in a mortar and pestle that had been cooled in liquid nitrogen. The fine powder was then homogenized with ten volumes of 0.1*M* $K_2HPO_4$-$KH_2PO_4$ buffer (*p*H 7.4) with a ground-glass homogenizer. The activity of BP hydroxylase, stable for at least 1 week in liquid nitrogen, was measured as follows (*7*). The reaction mixture contained 2 $\mu$mole of reduced nicotinamide adenine dinucleotide phosphate (NADPH), 3 $\mu$mole of $MgCl_2$, 0.5 ml of skin homogenate (containing 3 to 5 mg of protein), 100 nmole of BP in 0.05 ml of acetone, and 0.1*M* $K_2HPO_4$-$KH_2PO_4$ buffer (*p*H 7.4) in a total volume of 1.05 ml. The samples were incubated at 37°C for 30 minutes in air, and the amount of hydroxylated BP metabolites was measured (*7*). Since the activation and fluorescence spectra of BP metabolites formed by skin were identical to those of authentic 3-OH-BP in sodium hydroxide solution, the hydroxylation of BP was expressed as the number of picomoles of 3-OH-BP formed, even though the fluorescence measured may represent a mixture of phenolic metabolites. All results were corrected for recoveries (60 to 70 percent) that were obtained when 3-OH-BP was added to the skin homogenate and extracted as described above.

The activity of BP hydroxylase in human foreskin that had been cultured for 16 hours in control medium was low (about two to three times that of the blank), and culture of the skin for the same length of time in medium containing 10 $\mu M$ BA led to a two- to fivefold increase in activity of BP hydroxylase (Fig. 1). The increased enzyme activity in foreskins exposed to BA in the culture media could not be explained by a decreased breakdown of preexisting enzyme, as fresh foreskins had slightly less enzyme activity than did foreskins maintained for 16 hours in control media. The rate of BP hydroxylation observed with the foreskin homogenate was proportional to the concentration of the tissue (1 to 5 mg of protein), and the reaction rate was constant for at least 30 minutes. Maximum enzymatic activity required the presence of NADPH; little or no enzymatic activity could be detected if NADH was substituted for NADPH. In-

cubation in a nitrogen atmosphere, or in the presence of carbon monoxide, also resulted in little or no enzymatic activity. These results indicate that the skin enzyme requires oxygen and NADPH for its catalytic activity, as has been found for the liver microsomal enzyme system which hydroxylates BP (*1*).

Variation in the inducibility of BP hydroxylase obtained in skin samples from different newborn infants may, among other reasons, be due to genetic factors in the neonates, as well as to differences in exposure of the mother during gestation to drugs, environmental hydrocarbons, and so forth. Further studies will be necessary to evaluate the relative contributions of these factors to the inducibility of BP hydroxylase in human skin, and to determine whether the control amount and inducibility of this enzyme in skin will be predictive of the ability of humans to metabolize BP and other environmental carcinogens.

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## Interactions of Immunoglobulins G and M in the Detection of the Mammalian C-Type Virus Cross-Reactive Antigen

Abstract. *The mammalian C-type tumor viruses share an antigenic determinant, gs-3, located on the major internal polypeptide of the virion. Detection of this determined in gel diffusion assays by antiserums prepared in rats by immunization with rat tumor homogenates carrying murine virus and serums prepared in a rabbit by immunization with purified murine gs antigen depended on antibodies present in the fractions containing immunoglobulins M and G. The immunoglobulin G fraction by itself precipitated only the homologous murine antigen. Neither fraction alone precipitated heterologous (cat, rat, or hamster) antigen (definition of the gs-3 reaction), while a mixture of the two fractions did. The gs-3 reaction was eliminated by treatment of the serums with β-mercaptoethanol, also indicating a requirement for immunoglobulin M antibodies.*

The major internal protein (group-specific, gs, protein) of four mammalian C-type viruses carries both species-specific (gs-1) (*1*) and cross-reactive, interspecific (gs-3) (*2*) antigenic determinants (*3–5*). The strongest evidence for this conclusion derives from precipitation experiments with internally labeled, highly purified feline leukemia virus (FeLV) gs protein. In these experiments, both guinea pig antiserum to feline virus specific gs-1 and rat serum prepared against tumors carrying murine sarcoma virus and containing gs-3 antibody, precipitated 100 percent of the labeled antigen preparation (*6*). Nonetheless, in the course of analysis of antiserums prepared against purified gs protein or of antiserums from animals immunized with tumor homogenates containing murine sarcoma virus, occasional double preciptin lines were observed in gel diffusion assays even with highly purified antigen preparations. Among the possibilities considered to explain this observation was the variable occurrence of immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies in the various serums. Our data support this hypothesis and further show an unexpected synergism of IgM and IgG fractions for visualization of the gs-3 reaction in agar gel diffusion.

The various C-type viruses were purified from chronically infected tissue cultures, and the gs proteins were obtained by isoelectric focusing as described (*4, 5, 7, 8*).

The antiserums used in the present study were rabbit antiserum to the purified gs protein from murine C-type virus (MuLV) prepared by multiple inoculations of purified protein, and rat antiserum prepared by multiple injections of Fisher rats with homogenates of murine sarcoma virus–induced tumors transplanted in the Fisher strain (*3*).

The two antiserums and normal rat and rabbit serums were separated into IgM- and IgG-containing fractions by gel filtration with Sephadex G-200 (*9*). The buffer system used was 0.02*M* tris-HCl, *p*H 8.2, containing 0.2*M* NaCl. Reduction and alkylation of IgM fractions were carried out as follows. One volume of 1.0*M* β-mercaptoethanol was added to nine volumes of serum and the mixture was incubated for 2 hours at room temperature. Serums were then alkylated by dialysis against 0.02*M* iodoacetamide for 16 hours in the cold (*10*).

Gel diffusion was made on microscope slides with 0.8 percent agarose in 0.05*M* tris buffer, *p*H 7.4, containing 0.1*M* NaCl and merthiolate (1 : 10,000) as a preservative. Complement fixation (CF) was carried out by the microtiter procedure as used in this laboratory (*11*).

The ability of the rat antiserums (designated MSV-I) to detect gs-3 in gel diffusion assays has been reported (*3*). The immunization procedure results in essentially a 100 percent response to gs-3 determinants, whereas only a rare tumor-bearing animal gives adequate gs-3 antiserums (*2*). The rabbit antiserum against MuLV gs protein also detected the gs-3 determinant, as well as the MuLV gs-1 determinant, and in this respect differs at least quantitatively from guinea pig antiserums which were mainly reactive with gs-1 determinants (*3, 4, 5, 8*). However, as