ment, at rates of about 10^6 metric tons per day. At times when sediment discharges in the upper river are smaller (Fig. 2, E–G), the lower river resuspends sediment, again at rates of about 10^6 tons per day. This pattern tends to regulate the flow of suspended sediment to the Atlantic Ocean by keeping daily sediment discharges near the average value for large segments of the year.

On the basis of these measurements and taking into account the spacing of the measurements relative to an average annual hydrograph, we estimate the average discharge of suspended sediment past Óbidos to be between 3.0 and 3.5×10^6 metric tons per day. Therefore, the mean annual discharge of suspended sediment of the Amazon River at Óbidos is between 1.1 and 1.3×10^9 metric tons per year. This exceeds our previous estimate (*8*) by several hundred million tons per year and also agrees more closely with the measurements of sediment accumulation and transport on the continental shelf adjacent to the mouth of the Amazon (*9*).

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Monoclonal Antibody–Directed Radioimmunoassay Detects Cytochrome P-450 in Human Placenta and Lymphocytes

Abstract. *A multiplicity of cytochromes P-450 is responsible for the detoxification and activation of xenobiotics such as drugs and carcinogens. Individual differences in sensitivity to these agents may reside in the cytochrome P-450 phenotype. A monoclonal antibody–directed radioimmunoassay was developed that detects epitope-specific cytochromes P-450 in human placentas and peripheral lymphocytes. Placentas from women who smoked cigarettes contained greater amounts of cytochrome P-450 with the monoclonal antibody–specific epitope than placentas from nonsmokers. The amount of this cytochrome P-450 in human peripheral lymphocytes increased after treatment of the mitogenized lymphocytes with the cytochrome P-450 inducer benz[a]anthracene.*

The cytochromes P-450 are major enzymes for the metabolism of xenobiotics, such as drugs and carcinogens, as well as for certain classes of endobiotics, including steroids and prostaglandins (*1*). The cytochrome P-450–dependent metabolism of xenobiotics can lead to their detoxification or to their activation to toxic, mutagenic, or carcinogenic metabolites. The metabolic pathway depends in part on the phenotype or distribution of cytochrome P-450 isozymes in a tissue. The multiplicity of cytochromes P-450, the inability to measure directly their content in tissue, and their overlapping substrate- and product-specificity have hindered progress in elucidating the relation of the cytochrome P-450 phenotype to differences among individuals in the metabolism of drugs and carcinogens.

Monoclonal antibodies (MAb's) are useful for analysis of isozymic systems such as the cytochromes P-450. MAb's are chemically defined, homogeneous reagents that have epitope specificity and are easily and reproducibly obtained in large quantities (*2*). We have prepared and characterized panels of MAb's to several animal cytochromes P-450 (*3*). Use of the MAb's that inhibit catalytic activity has enabled us to identify immunochemically the cytochromes P-450 responsible for the metabolism of the carcinogen benzopyrene as measured by aryl hydrocarbon hydroxylase (AHH) and those responsible for the deethylation of the drug ethoxycoumarin as measured by ethoxycoumarin deethylase (ECD). In particular, we have used MAb 1-7-1, prepared to a highly purified rat liver cytochrome P-450 inducible by 3-methylcholanthrene, to measure the contribution of MAb-specific cytochrome P-450 to the total AHH and ECD activities in animal (*4*) and human (*5*, *6*) tissues. We have also shown the specificity of MAb 1-7-1 for individual cytochromes P-450 in immunopurification studies (*7*). This MAb has been useful in the radioim-

Table 1. Stability of AHH and measurements by radioimmunoassay (RIA) of cytochrome P-450 in human placenta and rat liver.

Treatment	Human placenta		Rat liver	
	AAH*	RIA†	AHH*	RIA†
None	61.2 (100)	100	2550.0 (100)	100
4°C for 48 hours	47.7 (78)	99	790.5 (31)	100
21°C for 24 hours	31.2 (51)	97	459.0 (18)	99

*Picomoles of 3-hydroxybenzo[*a*]pyrene per milligram of protein per minute; numbers in parentheses are percent of control. †Percent of maximum binding with 30 μg of placental microsomes or 0.3 μg of rat liver microsomes.

munoassay (*8*) of specific cytochromes P-450 in animal tissues.

The MAb 1-7-1 inhibits the activity of AHH in human placenta (*5*, *6*) and lymphocytes (*5*), which indicates that these human tissues and rat liver have AHH-active cytochromes P-450 that share a common epitope recognized by MAb 1-7-1. We now describe the use of MAb 1-7-1 to detect the presence of these cytochromes P-450 in human placenta and lymphocytes with a MAb-directed radioimmunoassay.

The MAb 1-7-1 was treated by reductive methylation (*9*) with ^{3}H-labeled sodium borohydride (New England Nuclear) to a specific activity of 1.9×10^6 count/min per microgram and was used in a solid-phase competitive radioimmunoassay (*8*). AHH activity was measured as described (*10*). The preparation and catalytic properties of the placental microsomes have been reported (*5*, *6*), as have the preparation of human lymphocytes and their induction with benz[*a*]anthracene (*5*).

Figure 1 illustrates competitive radioimmunoassay curves were calculated for several individual placenta samples. A standard curve for rat liver microsomes induced with 3-methylcholanthrene showed substantial inhibition of ^{3}H-labeled MAb 1-7-1 binding to wells coated with the microsomes (0.1 and 1 μg of rat liver microsomes reduced binding of the MAb by 25 and 86 percent, respectively) (Fig. 1). The placental microsomes inhibited binding to a lesser extent than the induced rat liver microsomes. Ten micrograms of placental microsomes from women who smoked cigarettes generally reduced MAb binding by 10 to 40 percent, whereas 50 and 100 μg inhibited up to 90 percent of the total MAb binding. In contrast, little inhibition (less than 10 percent) was observed for placental microsomes from women who did not smoke, even with 100 μg of microsomal protein. The greater degree of inhibition observed with placentas from smokers relative to those from nonsmokers indicates a higher amount of MAb-specific cytochrome P-450. The radioimmunoassay therefore successfully identified a cytochrome P-450 whose concentration is higher in placentas from smokers. In addition, the different competition curves obtained with placentas from different individuals were reproducible in independent measurements; this suggests the ability of the assay to detect individual variation in the amount of MAb-specific cytochrome P-450.

Two antigenically distinct forms of cytochrome P-450 were identified in human placentas (*5*, *6*). One form contains the epitope recognized by MAb 1-7-1, is responsible for the activities of AHH and ECD that are induced by smoking, and is sensitive to inhibition by MAb 1-7-1. The second form is responsible for the activity of ECD in placentas from nonsmokers as well as for the varying fractions (0 to 70 percent) of this activity in tissue from individual smokers that is insensitive to inhibition by MAb 1-7-1. Because the radioimmunoassay is based on MAb 1-7-1, it is a specific probe for a type of cytochrome P-450 responsible for the activities of AHH and ECD induced by smoking.

The activity of AHH is a common and generally used assay for cytochrome P-450 induced by polycyclic hydrocarbons.

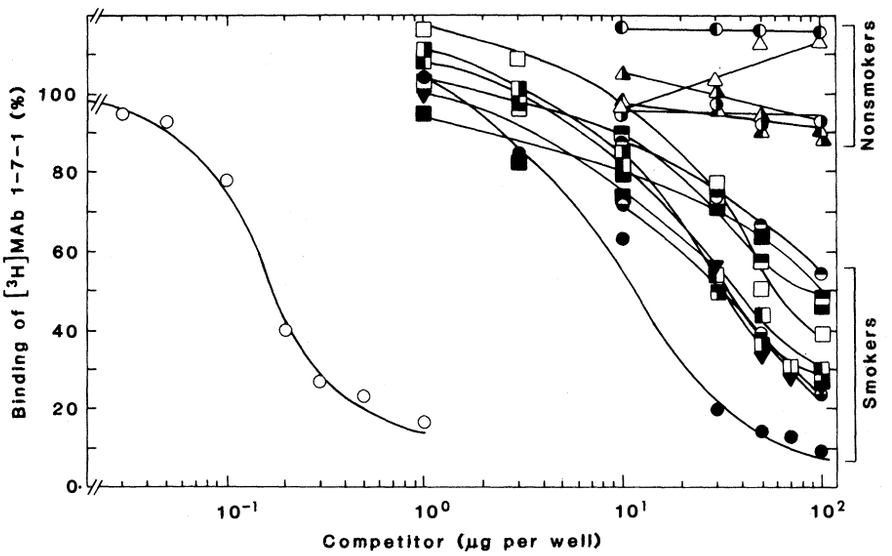

Fig. 1. Competitive radioimmunoassay for human placental microsomes with MAb 1-7-1. The microtiter plate (96 wells; Dynatech) was coated with rat liver microsomes induced with 3-methylcholanthrene (2 μg per well) and stored overnight at 4°C. ^{3}H-labeled MAb 1-7-1 [3691 count/min per well diluted in 5 m*M* phosphate-buffered saline (*p*H 7.4), 0.2 percent bovine serum albumin, and 0.6 percent 3-[(3-cholamidopropyl)dimethylammonio]-1-propane sulfonate] was incubated overnight at 4°C with different concentrations of rat liver microsomes treated with 3-methylcholanthrene (○) and placental microsomes from smokers and nonsmokers (each symbol represents an individual placenta as noted). The 100 percent binding level (1103 count/min per well) was obtained in the absence of competing antigens. The data are reported as means of duplicate determinations. The standard errors for the individual points averaged ±5.6 percent.

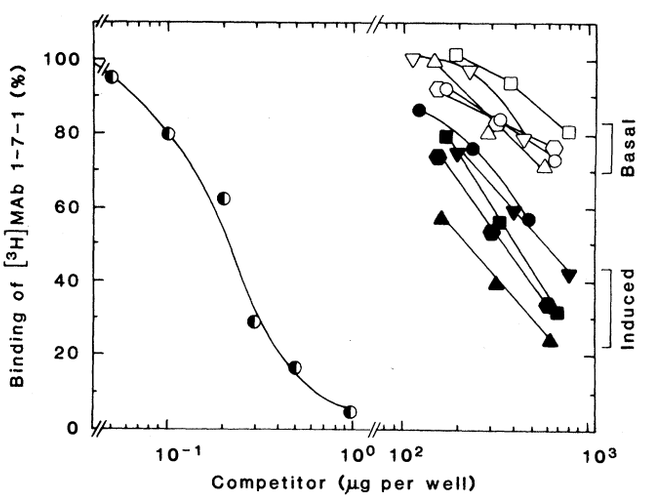

Fig. 2. Competitive radioimmunoassay for human lymphocytes with MAb 1-7-1. Lymphocytes from normal human donors were mitogenized with 1 percent phytohemagglutinin and 1 percent pokeweed mitogen for 48 hours. For induction, lymphocytes were treated for 16 hours with benz[*a*]anthracene (2 μg/ml) in 0.1 percent dimethyl sulfoxide. Untreated and induced lymphocytes were then washed, harvested, and homogenized. The assay was performed as described in the legend to Fig. 1, except that ^{3}H-labeled MAb 1-7-1 (6804 count/min per well) was incubated overnight with different concentrations of rat liver microsomes treated with 3-methylcholanthrene (◐), homogenates of uninduced lymphocytes (open symbols), and lymphocytes induced with benz[*a*]anthracene (closed symbols). Each open or closed symbol represents lymphocytes from a different individual. The 100 percent binding level (1993 count/min per well) was obtained in the absence of competing antigens. The data are reported as means of duplicate determinations. The standard errors for the individual points averaged ±1.4 percent.

However, this assay is not as specific as an MAb-directed radioimmunoassay because many different isozymes of cytochrome P-450, including constitutive forms, are AHH-active. To compare the usefulness of the radioimmunoassay and of the assay for AHH activity, we examined the stability of placental cytochrome P-450 as measured by the two methods (Table 1). Measurements were made with microsomes stored at 21°C or 4°C. The AHH activity for both samples declined substantially under the conditions of the test, but little change was observed in the radioimmunoassay measurements. The catalytic activity of the AHH-active cytochrome P-450 is therefore more unstable than the MAb-specific epitope detected by the radioimmunoassay. This assay is therefore both more reliable and more specific than measurements of catalytic activity of cytochrome P-450 in tissues.

We next applied the radioimmunoassay to lymphocytes, which are more readily available than placentas and thus more useful for studies of biochemical epidemiology and for phenotyping human tissues. Basal lymphocytes and those induced with benz[*a*]anthracene from different individuals were examined. Blood samples (50 ml) were obtained from each individual; these yielded enough lymphocytes for duplicate assays with different amounts of cell homogenate. Treatment with benz[*a*]anthracene resulted in a greater amount of MAb-specific cytochrome P-450, as revealed by the radioimmunoassay (Fig. 2). Because only about 6 ml of blood is needed per duplicate measurement of both basal and induced cells, this assay is well suited for screening studies of humans.

A relation between the inducibility of AHH in lymphocytes and the incidence of lung cancer has been suggested, but the data are conflicting (*11*). The various results of previous studies (*12*) may be due to differences in sample handling or possible loss of enzyme activity during preparation. Our MAb-based assay may help in resolving the controversy because it is rapid and sensitive and bypasses these problems. It may be more applicable for screening studies of appropriate populations and a more suitable and specific method than the conventional enzyme assay, especially for detecting human cytochromes P-450 that are unstable or present at low levels of enzyme activity.

The specificity of MAb's to individual epitopes makes them useful for detecting single enzymes or classes of enzymes within a system comprising multiple enzymes. The cytochromes P-450 are a paradigm of such a system and are also the enzymatic interface between xenobiotics and higher organisms and because they metabolize endobiotics. The cytochrome P-450 profile of a tissue may determine differences within an individual in the metabolism of cytochrome P-450 substrates. The MAb-directed radioimmunoassay for cytochrome P-450 should also be useful for relating tissue cytochrome P-450 phenotype and drug and carcinogen sensitivity and for detecting polymorphisms.

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Two Types of Somatostatin Receptors Differentiated by Cyclic Somatostatin Analogs

Abstract. *Somatostatin receptors in rat brain, pituitary, and pancreas were labeled with two radioiodinated analogs of somatostatins 14 and 28. Two cyclic analogs of somatostatin, SMS201–995 and cyclo(Ala-Cys-Phe-D-Trp-Lys-Thr-Cys), showed biphasic displacement of binding to somatostatin receptors by these radioligands. In contrast, all other somatostatin analogs, including somatostatin-14, competed for the receptor sites with monophasic displacement of radioligand receptor binding. Thus two types of somatostatin receptors were identified. It was found that the pituitary and pancreas have predominantly one type of somatostatin receptor whereas the brain has both, and that different regions of the brain have various proportions of the two types. These findings suggest methods to characterize other types of somatostatin receptors subserving somatostatin's diverse physiological functions, including a potential role in cognitive function and extrapyramidal motor system control.*

Somatostatin is a tetradecapeptide widely distributed in the mammalian central nervous system and peripheral tissues where it exerts a wide variety of physiological effects (*1*). Somatostatin concentration is markedly increased in the basal ganglia in Huntington's disease (*2, 3*), whereas it is decreased in the cortex in Alzheimer's disease (*4*). Thus somatostatin may play a significant role in extrapyramidal motor system and cognitive functions.

Somatostatin stimulates release of [^{3}H]dopamine from the striatum in vivo and in vitro (*5*) and increases dopamine turnover rates without affecting other biogenic amines (*6*). These effects are probably mediated through specific somatostatin receptors. Somatostatin receptors in the rat have been studied in great detail (*7–10*) by using binding to brain membranes. An autoradiographic study (*11*) showed that somatostatin receptors are preferentially distributed in the ventral medial portion of the caudate nucleus compared to its dorsolateral region. Dopamine turnover is also highest in the nucleus accumbens and ventromedial striatum (*12*), and dopamine receptors follow the same distribution (*13*), suggesting a possible interaction between dopamine and somatostatin. So-