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Target Cells for the Polychlorinated Biphenyl Metabolite 4,4′-Bis(methylsulfonyl)-2,2′,5,5′-tetrachlorobiphenyl

Characterization of High Affinity Binding in Rat and Mouse Lung Cytosol

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SUMMARY

When a tritium-labeled metabolite of polychlorinated biphenyls (PCB), 4,4′-bis([^{3}H]methylsulfonyl)-2,2′,5,5′-tetrachlorobiphenyl [(^{3}H-MeSO$_2$)$_2$TCB] is administered intraperitoneally to rats, a selective labeling is registered in the apical cytoplasm of the nonciliated bronchiolar (Clara) cells of the lung as determined by microautoradiography of sections of methacrylate-embedded tissue. *In vitro*, (^{3}H-MeSO$_2$)$_2$TCB binds with high affinity (K_d = 2.5–15 nM) and high capacity (B_{max} = 30–70 pmol/mg of protein) to rat lung cytosol. Binding of (^{3}H-MeSO$_2$)$_2$TCB to the high affinity sites is temperature dependent, reversible, and saturable. The sites seem to reside within a protein-like component, since proteolytic enzymes significantly reduce the binding. Physicochemical characterization of the (^{3}H-MeSO$_2$)$_2$TCB-binding protein indicates a Stokes radius of 22 Å and a sedimentation coefficient of 1.7 S and, on the basis of these parameters, an apparent molecular weight of 16,000 may be calculated. The binding entity has an apparent pI of 5.3 and elutes as a single radioactive peak from CM-Sepharose at 75 mM acetate. Binding with similar affinities (IC_{50} values, 4–65 nM) is shown to occur also with other PCB methyl sulfones, whereas only one PCB, 2,2′,4,4′5,5′-hexachlorobiphenyl, competes for (^{3}H-MeSO$_2$)$_2$TCB binding, but with a lower affinity (IC_{50} = 3 μM). Among other compounds tested, only progesterone and some derivatives thereof display an affinity for the (^{3}H-MeSO$_2$)$_2$TCB-binding protein (IC_{50} values ranging from 1 to 10 μM). Lung cytosol shows by far the highest amount of specific (^{3}H-MeSO$_2$)$_2$TCB binding. However, low but detectable amounts are also found in cytosolic preparations from prostate, kidney, and large intestine. Finally, (^{3}H-MeSO$_2$)$_2$TCB also binds to an entity in mouse lung cytosol with the same physicochemical characteristics as that in rat lung cytosol and to a progesterone-binding protein purified from rabbit uterus (uteroglobin). It is concluded that rat lung contains a uteroglobin-like macromolecule with a pronounced affinity for at least certain PCB methyl sulfones, and it is suggested that this binding entity is responsible for the striking accumulation of such metabolites in lung tissue following administration of PCB to rats and mice.

INTRODUCTION

Polychlorinated biphenyls are industrial chemicals which have become widespread environmental pollutants. As members of the group of compounds referred to as halogenated aromatic hydrocarbons, they have received much attention over the last two decades due to the characteristic toxic symptoms they produce and their ability to induce a whole battery of enzymes, including the microsomal monooxygenase aryl hydrocarbon hydroxylase (for reviews, see Refs. 1 and 2). The potency of different PCB[1] congeners to elicit toxic and biochem-

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[1] Abbreviations and trivial names are PCB, polychlorinated biphenyl; (MeSO$_2$)$_2$TCB, 4,4′-bis(methylsulfonyl)-2,2′,5,5′-tetrachlorobiphenyl; diethylstilbestrol, *trans*-3,4-bis(hydroxyphenyl)-3-hexene; estrone, 1,3,5,(10)-estratrien-3-ol-17-one; 17β-estradiol, 1,3,5(10)-estratriene-3,17β-diol; corticosterone, 4-pregnene-11β,21-diol-3,20-dione; dexamethasone, 1,4-pregnadiene-9-fluoro-16α-methyl-11β,17α,21-triol-3,20-dione; testosterone, 4-androsten-17β-ol-3-one; 5α-dihydrotestosterone, 5α-androstan-17β-ol-3-one; progesterone, 4-pregnene-3,20-dione; R5020, 17α,21-dimethyl-19-nor-4,9,pregnadiene-3,20-dione; DCC, dextran-coated charcoal; BSA, bovine serum albumin; R_s, Stokes radius (Å).

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ical responses varies greatly. Also the susceptibility of different tissues to the action of PCB and related compounds varies from species to species. Although much work has been carried out on the structure-activity relationships with regard to aryl hydrocarbon hydroxylase induction and toxic response (reviewed in Ref. 1), little attention has been paid to the molecular mechanisms determining the tissue and species selectivity.

Sulfur-containing metabolites of PCB have been demonstrated in both mice and rats (3, 4). The metabolic pathways leading to the generation of these metabolites have been shown to involve glutathione conjugation of an arene oxide intermediate (5), to require the presence of an intact intestinal microflora (6), and to give rise to PCB methyl sulfones with a marked affinity for the bronchial mucosa and the kidney cortex (6, 7). This is especially interesting in light of the respiratory symptoms observed in patients from the Yusho incidence in Japan 1968, a mass food poisoning caused by the ingestion of a commercial brand of rice oil contaminated with PCB (1, 8). Several patients still display these respiratory symptoms, more than 10 years after the accident (9).

In the present paper, we describe the application of microautoradiography to determine the cells responsible for the *in vivo* accumulation of (^{3}H-MeSO$_2$)$_2$TCB and an initial characterization of the possible molecular mechanism underlying the great selectivity in distribution of (MeSO$_2$)$_2$TCB *in vivo* is given.

MATERIALS AND METHODS

Chemicals. (^{3}H-MeSO$_2$)$_2$TCB (specific activity, 4 and 16 Ci/mmol) and unlabeled (MeSO$_2$)$_2$TCB were synthesized as described previously (10). The unlabeled 2,4′,5-trichlorobiphenyl, 2,2′,5,5′-tetrachlorobiphenyl, and 2,2′,4,5,5′-pentachlorobiphenyl were synthesized according to the Cadogan aryl coupling reaction (11). 2,2′,4,4′,5,5′-Hexachlorobiphenyl was prepared by the Ullman procedure (12). 4-Methylsulfonyl-2,2′,5,5′-tetrachlorobiphenyl was synthesized from 4-nitro-2,5-dichloroaniline and 1,4-dichlorobenzene by the route described by Bakke *et al.* (4). 4-Methylsulfonyl-2,3′,4′,5-tetrachlorobiphenyl and 4-methylsulfonyl-2,2′,4′,5,5′-pentachlorobiphenyl were synthesized as reported elsewhere (see Ref. 10 in Ref. 6). Pentachlorobenzthiol was methylated and the sulfide obtained was oxidized in order to give methylsulfonylpentachlorobenzene. 3-Methylsulfonyl-2,2′-bis(4-chlorophenyl)-1,1-dichloroethylene was synthesized in a Friedel Craft-type acylation (13). The unlabeled compounds were all >99.5% pure according to gas chromatography. 2,3,7,8-Tetrachlorodibenzofuran was kindly supplied by Dr. C. Rappe (Umeå, Sweden). Benzo(*a*)pyrene, 3-methylcholanthrene, diethylstilbestrol, estrone, 17β-estradiol, corticosterone, dexamethasone, testosterone, and 5α-dihydrotestosterone were purchased from Sigma Chemical Co. (St. Louis, MO). Progesterone was obtained from the Upjohn Co. (Kalamazoo, MI). 5α-Pregnane-3,20-dione and 5β-pregnane-3,20-dione were purchased from Steraloids Inc. (Wilton, NH).

Sephadex G-75, CM-Sepharose, Dextran T-70, Blue Dextran 2000, Polybuffer, and polybuffer exchanger (PBE 94) were obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). Ready made thin layer polyacrylamide gels for isoelectric focusing (pH 3.5–9.5) were purchased from LKB-Produkter AB (Bromma, Sweden). Lipidex 1000 and Scintillator 299 were obtained from Packard Instruments Co. (Downers Grove, IL). Trypsin and calf thymus DNA were obtained from Worthington Biochemical Corp. (Freehold, NJ) and papain, pronase, deoxyribonuclease I, and ribonuclease A were from Boehringer (Mannheim, F. R. G.). Activated charcoal[2] (Norit A), 2-hydroxyethylmercaptan, cytochrome *c* (equine heart), myoglobin (equine heart), deoxyribonuclease I (bovine pancreas), ovalbumin (hen eggwhite), albumin (bovine serum), and aldolase (rabbit muscle) were purchased from Sigma.

Uteroglobin from rabbit uterus was a kind gift from Dr. E. Milgrom, INSERM U 135, Bicetre, France and Dr. O. Jänne, The Population Council, New York. All other chemicals were analytical grade products from Sigma or Merck A. G. (Darmstadt, F. R. G.).

Buffers. The following are buffer compositions: buffer A: 20 mM potassium phosphate, 1 mM EDTA, 10% (w/v) glycerol, 2 mM 2-hydroxyethylmercaptan, pH 7.2; buffer B: 25 mM sodium acetate, 1 mM EDTA, pH 4.5; buffer C: 150 mM sodium acetate, 1 mM EDTA, pH 7.0.

Animals. Male Sprague-Dawley rats (180–220 g) obtained from Anticimex, Stockholm were used. In some experiments, male C57BL/6J mice (21 g) obtained from Jackson Laboratories (Bar Harbor, ME) were used.

Wet-mounting microautoradiography with methacrylate-embedded tissue. Rats were injected intraperitoneally with (^{3}H-MeSO$_2$)$_2$TCB (375 μCi; 98–167 nmol/kg) dissolved in dimethyl sulfoxide (100 μl) and killed 2, 3, or 6 days after injection. The lungs were fixed by intratracheal inflation of 0.1 M phosphate buffer (pH 7.2) containing 1.5% (w/v) glutaraldehyde and 1.5% (w/v) formaldehyde. After postfixation in the same fixative, pieces of lung tissue were washed in a phosphate buffer (pH 7.2) and dehydrated in an ethanol series before being embedded in hydroxyethyl methacrylate (Sorvall embedding medium, DuPont Co., Newton, CT). One-μm-thick sections were cut and dipped in NTB-2 liquid film emulsion (Eastman-Kodak, Inc., Rochester, NY). The autoradiograms were developed after 10–20 days of exposure (2°) and the sections were stained with toluidine blue or hematoxylin-eosin. Some of the sections were stained with periodic acid-Schiff-hematoxylin before being dipped in the liquid film emulsion.

Preparation of cytosol. Rats and mice were killed by cervical dislocation and the lungs were immediately removed onto ice. All successive work was carried out at 0–4°. The lungs were finely minced and homogenized in 2 volumes of buffer A in a Teflon/glass Potter-Elvehjem homogenizer. The homogenate was then centrifuged at 105,000 × *g* for 60 min. The supernatant, hereafter termed cytosol, was removed with care taken to avoid contamination with the lipid layer. Aliquots were stored at −70° for up to 6 months without any appreciable loss of binding capacity. The protein content of the cytosolic preparations was determined according to Lowry *et al.* (14).

Cytosols from other tissues were prepared as described above, with the following exceptions. (a) The small and large intestines were flushed with ice-cold potassium chloride (0.15 M) and cut open longitudinally; the mucosa was scraped off with a glass microscope slide on a glass plate and then homogenized and centrifuged as above. b) The skeletal muscle tissue and the heart were frozen and powderized in liquid nitrogen in a prechilled mortar according to Dahlberg *et al.* (15). Thereafter, buffer A was added and the homogenate was centrifuged at 105,000 × *g* for 60 min.

Binding assay. Buffer A (0.5 ml) containing BSA (1 mg) in the presence or absence of lung cytosolic protein (15–50 μg) was incubated with the appropriate amount of (^{3}H-MeSO$_2$)$_2$TCB dissolved in ethanol (10–20 μl) for the indicated periods of time. Regardless of the incubation temperature, the tubes were placed on ice before terminating the incubation by the addition of 0.5 ml of DCC (0.5%, 0.5 g of charcoal and 0.05 g of Dextran T70 per 100 ml of buffer A). The incubations were agitated and left on ice for 10 min. The charcoal was then pelleted by centrifugation at 1,500 × *g* for 10 min. The supernatant was assayed for radioactivity. To determine correctly the total radioactivity added, aliquots (10–20 μl) of the incubations were removed shortly before the addition of the DCC. In some experiments, the cytosolic protein from rat lung was replaced with cytosolic protein from mouse lung or with uteroglobin.

[2] Prior to use, the charcoal was repeatedly washed with glass-distilled water to remove slowly sedimenting particles by decantation and finally washed with ethanol before drying.

The affinity of other ligands for the (^{3}H-MeSO$_2$)$_2$TCB-binding protein in rat lung cytosol was tested as above with a fixed amount of lung cytosolic protein (15 μg/0.5 ml), 15 nM (^{3}H-MeSO$_2$)$_2$TCB, and varying concentrations of unlabeled compounds added in small volumes of dioxane (10 μl) or ethanol (15 μl). Vehicle alone was added to obtain values for total binding.

Association and dissociation kinetics. Duplicate incubations containing BSA (2 mg) and lung cytosolic protein (50 μg) per ml of buffer A for each of the temperatures to be tested were kept on ice, (^{3}H-MeSO$_2$)$_2$TCB was added to a concentration of 20 nM, the duplicates were rapidly transferred to water baths with the preset temperatures, and the time was set to zero. At various time points, aliquots were removed onto ice and the binding was assayed as described above. For control of nonspecific binding, duplicate incubations containing BSA as the only protein were run in parallel.

To demonstrate the reversibility of the binding and to follow the rate of dissociation, association at 37° was first performed as described. When maximum association had been achieved, the incubations were divided into two equal parts, one of which received a 100-fold molar excess of unlabeled (MeSO$_2$)$_2$TCB and the other vehicle only. Aliquots were then removed at different intervals and treated as above.

Saturation analysis. The saturability of the (^{3}H-MeSO$_2$)$_2$TCB binding was studied by incubating fixed amounts of cytosolic protein (15–50 μg/ml) with (^{3}H-MeSO$_2$)$_2$TCB concentrations ranging from 0–100 nM, using the binding assay described above. These samples were incubated to equilibrium at different temperatures, and all K_d and B_{max} values were calculated by linear regression analysis of data obtained by Scatchard plots (16).

Tissue distribution. In order to have sufficient amounts of protein present to detect even low levels of specific (^{3}H-MeSO$_2$)$_2$TCB binding, the experiments were performed with cytosolic preparations at a protein concentration of 2 mg/ml of buffer A. To saturate this amount of protein in lung cytosol would consume excessive amounts of radioactivity, and a lowering of the specific activity would decrease the sensitivity of the assay. These experiments were thus performed with a (^{3}H-MeSO$_2$)$_2$TCB concentration (10 nM) that did not saturate all the specific (^{3}H-MeSO$_2$)$_2$TCB-binding sites in lung cytosol. The binding experiments were performed at both 0° and 37°, aliquots were removed at different intervals, and the binding was assayed as described.

Enzymatic digestion. Triplicates of cytosolic protein (15 μg/0.4 ml of buffer A containing 2 mM $CaCl_2$) were incubated with different hydrolytic enzymes (0–500 μg) for 30 min at 37°. BSA (1 mg in 0.1 ml of buffer A) was then added to each incubation along with (^{3}H-MeSO$_2$)$_2$TCB to a final concentration of 15 nM, and the samples were incubated for another 30 min at 37° before being assayed as above. Appropriate blanks without hydrolytic enzymes were used to calculate 100% specific binding, and the effects of the enzymes were expressed as percentage of control specific binding remaining after treatment.

Gel permeation chromatography and density gradient centrifugation. Sephadex G-75 was equilibrated in buffer A containing 0.02% (w/v) sodium azide and packed into siliconized columns (2.6 × 65 cm). The columns were calibrated with Blue Dextran 2000, cytochrome *c*, myoglobin, DNase I, ovalbumin, and [^{14}C]glucose. The flow rate was 1.9 ml/cm^2/hr, the sample volume was less than 2% of the total column volume, and 4-ml fractions were collected. The calculations of molecular parameters were performed according to Porath (17) and to Siegel and Monty (18). The Stokes radii were determined graphically from a plot of −log [Stokes radius] versus K_d ($K_d = (V_e - V_0)/(V_t - V_0)$), where K_d is the distribution coefficient, V_e is the elution volume, and V_0 and V_t are the void and the total liquid volumes of the column, respectively.

Aliquots of labeled cytosol (0.2 ml) were layered onto linear 5–20% (w/v) sucrose gradients and centrifuged in an SW 60 Ti rotor using the $\omega^2 dt$-integrator in a Beckman L8-M centrifuge set for 1.8×10^{12} rad^2/sec (corresponding to approximately 300,000 × *g* for 15 hr). Fractions were then collected by puncturing the bottom of the tubes. ^{14}C-labeled standard proteins prepared according to Rice and Means (19) were run both as internal standards and on separate gradients in order to determine approximate S values for the (^{3}H-MeSO$_2$)$_2$TCB-labeled peaks.

Isoelectric focusing in polyacrylamide gels and chromatofocusing. Ready-made polyacrylamide gels were prefocused for 10 min using an LKB 2117 Multiphor instrument (Stockholm, Sweden). The maximum settings of the LKB 2103 power supply were 20 mA, 1200 V, and 18 W. An LKB 2209 Multitemp cryothermostat supplied ethanol at −2°, which was then pumped through the cooling plate. The samples were applied in acrylic plastic frames (inner dimension, 7 × 15 mm; height, 3 mm) placed on the gel 1 cm from the cathode strip. Eight samples were analyzed simultaneously with hemoglobin and ferritin as standards between each sample frame. The electrofocusing was carried out for 105 min at 2°, under the same conditions as the prefocusing. The pH was measured in the middle of the gel at 1-cm intervals after the electrofocusing at 2°, using a surface electrode (type 403-30, Ingold, Zurich, Switzerland). In front of each sample frame, sections measuring 30 mm across were cut from the gel and sliced into 3-mm sections with a pair of scissors. The slices were placed into plastic scintillation vials and the radioactivity was extracted in the scintillation fluid overnight before it was assayed.

Chromatofocusing was performed on PBE 94 polybuffer exchanger packed in a siliconized column (1.0 × 15 cm) and equilibrated in imidazole HCl (25 mM, pH 7.4). The column was eluted with Polybuffer 74 in a 1:8 dilution with water and the pH was adjusted to 4. The flow rate was 15.7 ml/cm^2/hr, and 3-ml fractions were assayed for pH and radioactivity.

Cation exchange chromatography. CM-Sepharose was equilibrated in buffer B and packed into a siliconized column (2.6 × 8.0 cm). The column was washed with buffer B at 0–4° until both the conductivity and pH values were identical with those of the washing (=starting) buffer. Lung cytosol was extensively dialyzed against buffer B at 0–4°. Before labeling with (^{3}H-MeSO$_2$)$_2$TCB (20 nM), any precipitated protein was pelleted (the final protein concentration was 5 mg/ml). The (^{3}H-MeSO$_2$)$_2$TCB-binding protein did not precipitate at pH 4; instead, it was found to be very stable in the acidic pH range and bound the tritiated ligand as efficiently at pH 4 as at 7.2 (data not shown). (^{3}H-MeSO$_2$)$_2$TCB-labeled cytosol (6 ml) in buffer B was applied on the column and washed with 3 column volumes of buffer B. The radioactive material bound was then eluted with a linear acetate and pH gradient prepared by the mixing of equal volumes of buffer B and buffer C. The flow rate was 3.8 ml/cm^2/hr, and 3-ml fractions were collected.

Liquid scintillation counting. Radioactivity was measured in Scintillator 299 in an LKB-Wallac 1216 Rackbeta II scintillation spectrometer with an average counting efficiency of 40%.

RESULTS

In vivo distribution of (^{3}H-MeSO$_2$)$_2$TCB. Whole-body autoradiograms of rats injected intraperitoneally with (^{3}H-MeSO$_2$)$_2$TCB were dominated by a strong labeling of the lung and of the kidney cortex (data not shown). There was also an accumulation of radioactivity in the colorectal mucosa and in a restricted area located in the dorsal part of the rat ventral prostate (data not shown). A microautoradiogram of a metacrylate-embedded section of a rat lung is shown in Fig. 1. A strong labeling was observed in specific cells of the bronchiolar epithelium. The cells lacked cilia and were characterized by a cytoplasm projecting into the bronchiolar lumen. These nonciliated bronchiolar cells therefore appear to correspond to the so-called Clara cells. The labeled Clara cells were most abundant in the bronchioles, where they comprised ~50% of the epithelial cells, but were also present in the larger airways. The silver grains were always present in the apical region of the cytoplasm bulging into

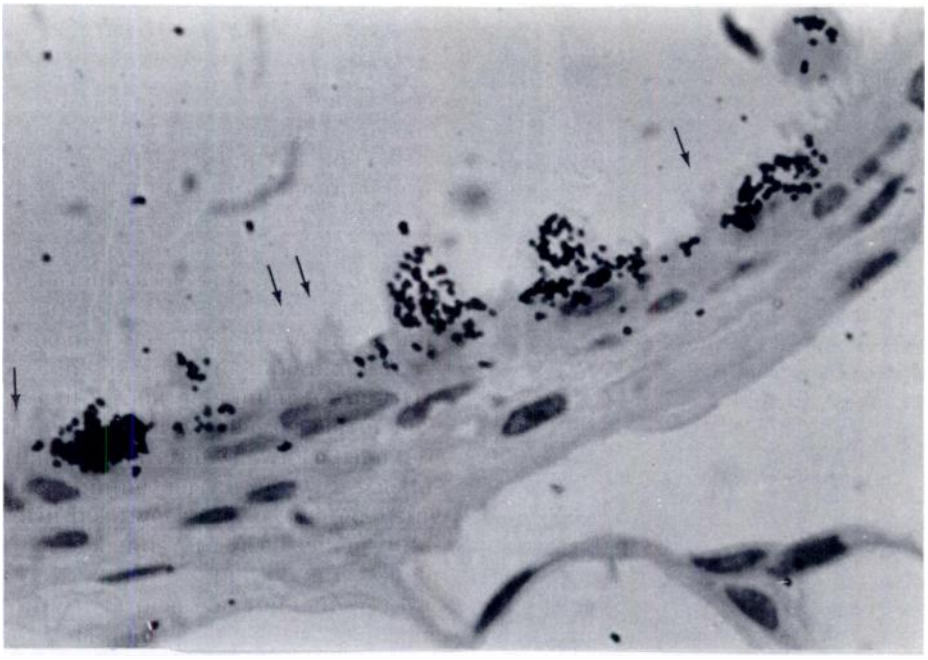

FIG. 1. *Microautoradiogram of a methacrylate section from a rat lung*
Rats were injected intraperitoneally with (^{3}H-MeSO$_2$)$_2$TCB (375 μCi; 98–167 nmol/kg in 100 μl of dimethyl sulfoxide) and killed after 2, 3, and 6 days. Microautoradiography of the lungs was performed as described in Materials and Methods. Black silver grains are selectively present over the Clara cells, whereas adjacent ciliated (arrows) bronchiolar cells contain no radioactivity (6 days postinjection, periodic acid-Schiff, 1470×).

the lumen and the labeling of the basal cytoplasm was generally low. The number of silver grains over the adjacent ciliated cells was low and generally not above background level.

In vitro binding assay. Initially, we developed an *in vitro* binding assay which allowed us to separate bound from unbound (^{3}H-MeSO$_2$)$_2$TCB. The assay was designed to use low amounts of cytosolic protein since initial experiments indicated a high capacity for binding in the lung cytosol. The use of DCC to separate unbound from bound steroids is a well established method. One of its major drawbacks is its tendency to adsorb not only unbound ligand, but also protein when used at low protein concentration. In order to avoid this problem, protecting proteins may be added to the solutions provided these proteins show only low binding of the ligand studied.

The results of experiments with solutions of increasing concentration of BSA and a fixed concentration of (^{3}H-MeSO$_2$)$_2$TCB (20 nM) are shown in Fig. 2. Only a low binding of (^{3}H-MeSO$_2$)$_2$TCB was seen, and the binding was nonspecific as determined by the inability of a 100-fold molar excess of unlabeled (MeSO$_2$)$_2$TCB to displace binding of (^{3}H-MeSO$_2$)$_2$TCB. However, when small amounts of lung cytosolic protein (50 or 100 μg) were added, the binding was dramatically increased (Fig. 2). Addition of lung cytosolic protein up to 100 μg showed only an increase in specific binding since the binding in the presence of a 100-fold molar excess of unlabeled ligand did not differ from the binding to BSA only. Fig. 2 also shows the necessity of a minimum BSA concentration of 1–2 mg/ml to avoid adsorption of lung cytosolic protein to the charcoal. In the following experiments, the

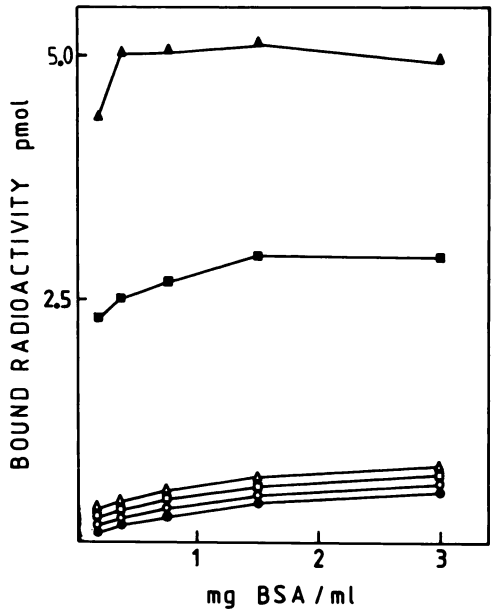

FIG. 2. *Binding of (^{3}H-MeSO$_2$)$_2$TCB to lung cytosol and BSA*
Increasing amounts of BSA (0–3 mg/ml of buffer A) were incubated with 20 nM (^{3}H-MeSO$_2$)$_2$TCB in the presence (○) or absence (●) of a 100-fold molar excess of (MeSO$_2$)$_2$TCB for 1 hr at 37°. The incubations (0.5 ml) were placed on ice, equal volumes of 0.5% (w/v) DCC were added, and the incubation mixtures were agitated and left on ice for 10 min. The charcoal was then pelleted and the supernatant was assayed for radioactivity. Parallel incubations (0.5 ml) were performed with the addition of 50 μg of lung cytosolic protein/incubation in the presence (□) or absence (■) of excess unlabeled (MeSO$_2$)$_2$TCB and also with the addition of 100 μg of lung cytosolic protein in the presence (△) or absence (▲) of excess unlabeled (MeSO$_2$)$_2$TCB. Each value represents the mean of triplicate determinations.

binding of (^{3}H-MeSO$_2$)$_2$TCB to 15–100 μg of lung cytosolic protein/ml in the presence of 2 mg of BSA/ml will be designated "total binding," whereas the corresponding binding in the absence of lung cytosolic protein will be designated "nonspecific" binding; thus, the difference constitutes the "specific binding."

Association and dissociation kinetics. The specific binding of (^{3}H-MeSO$_2$)$_2$TCB to lung cytosol (52 μg/ml) at different temperatures is shown in Fig. 3. At 37°, maximum association was reached after 60 min and then slowly declined, so that after 24 hr only 5% of the specific binding remained. At 20°, maximum association was reached after 4 hr and the specific binding remained quite stable for 24 hr. At 72 hr, 63% of the specific binding still remained. The kinetics at 10° were very similar to those at 20°, with maximum association after 8 hr and 76% remaining at 72 hr. At 0°, the association rate was slow; it took more than 24 hr to reach maximum specific binding, and no detectable inactivation was seen after 72 hr. It appears probable that the significant decrease in binding of (^{3}H-MeSO$_2$)$_2$TCB to lung cytosol at 37° and to a lesser extent at 10° and 20° is due to protein denaturation.

In a separate experiment, association was performed at 37° until maximum association was reached (Fig. 4). The addition of unlabeled (MeSO$_2$)$_2$TCB caused a pronounced dissociation of bound (^{3}H-MeSO$_2$)$_2$TCB, where 38% of the specific (^{3}H-MeSO$_2$)$_2$TCB binding remained 30 min after the addition of unlabeled (MeSO$_2$)$_2$TCB, demonstrating the reversibility of binding (Fig. 4).

When the association kinetics data were plotted as ln $[B_{eq}/(B_{eq} - B)]$ versus time, where B is the amount of specific (^{3}H-MeSO$_2$)$_2$TCB binding at time t and B_{eq} is the amount bound at equilibrium, the data yielded straight lines indicating a bimolecular reaction (data not shown). However, more detailed kinetic studies will require the use of a more rapid binding assay than the DCC technique and such studies will therefore have to await the development of such an assay.

Saturation and Scatchard analysis. The standard binding assay was used to obtain equilibrium binding data for lung cytosol (30 μg of protein/ml) incubated with 0–40 nM (^{3}H-MeSO$_2$)$_2$TCB for 60 min at 37° or for 24 hr at 0–4°. Fig. 5 shows the data from an experiment performed at 0–4° for 24 hr. The lung cytosolic protein was saturated at a concentration of 20 nM radioactive ligand. Based on the saturation analysis data shown in Fig. 5, calculation according to Scatchard (16) gave a B_{max} of 57

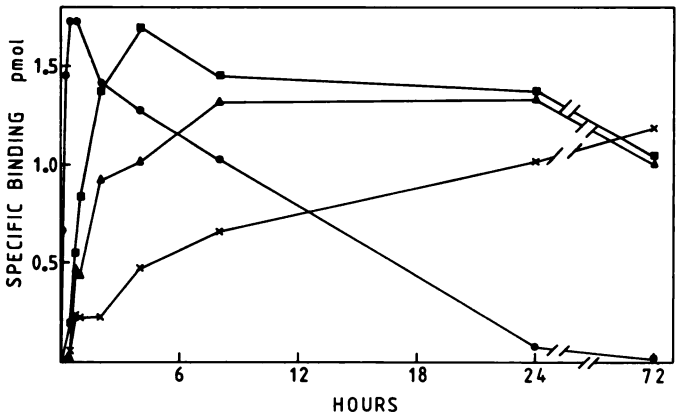

FIG. 3. *Time course of specific (^{3}H-MeSO$_2$)$_2$TCB binding to rat lung cytosol at various temperatures*

Rat lung cytosol (50 μg of protein/ml) in buffer A was incubated with 20 nM (^{3}H-MeSO$_2$)$_2$TCB at 0–4° (×), 10° (▲), 20° (■), and 37° (●), and the specific binding at various timepoints was determined as described in Materials and Methods. Each value represents the mean of duplicate determinations in two separate experiments.

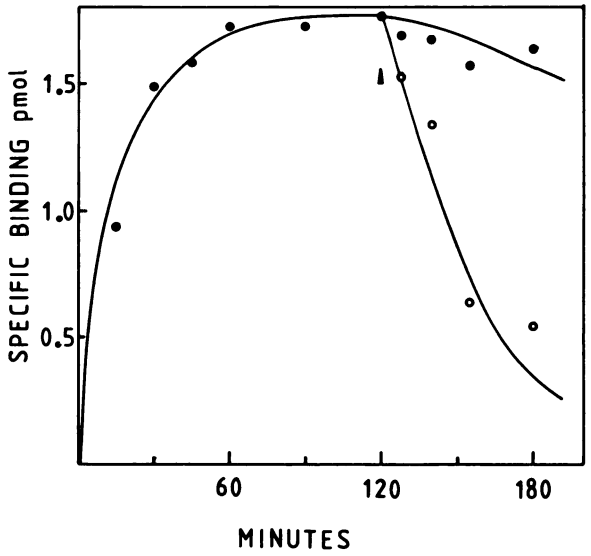

FIG. 4. *Reversibility of the specific (^{3}H-MeSO$_2$)$_2$TCB binding*

Rat lung cytosol (50 μg of protein/ml) in buffer A was incubated with 20 nM (^{3}H-MeSO$_2$)$_2$TCB at 37°, aliquots were removed at various timepoints, and the specific binding was determined as described in Materials and Methods. The arrow indicates the timepoint at which the incubations were divided into two parts, one set receiving a 100-fold molar excess of unlabeled (MeSO$_2$)$_2$TCB (○) and the other vehicle only (●). Each value represents the mean of triplicate determinations.

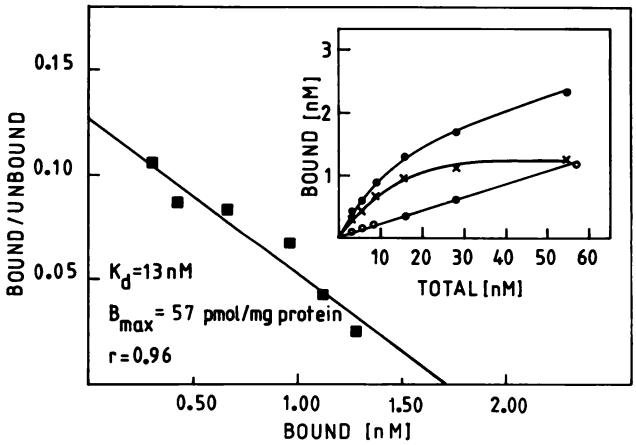

FIG. 5. *Saturation and Scatchard analysis of the binding of (^{3}H-MeSO$_2$)$_2$TCB to rat lung cytosol*

Rat lung cytosol (30 μg of protein/ml) in buffer A was incubated with increasing concentrations of (^{3}H-MeSO$_2$)$_2$TCB (0–60 nM) at 0–4° for 24 hr and the binding was then determined as described in Materials and Methods. Each value is the mean of triplicate determinations, and the data shown are representative of five such experiments. The inset shows a plot of the saturation analysis data, where the specific (^{3}H-MeSO$_2$)$_2$TCB binding (×) represents the difference between total (●) and nonspecific (○) binding [(^{3}H-MeSO$_2$)$_2$TCB binding to BSA alone]. From these data, a Scatchard plot (16) was constructed.

TABLE 1

Effect of enzyme treatment on specific (^{3}H-MeSO$_2$)$_2$TCB binding

Rat lung cytosol (15 μg in 0.4 ml of buffer A containing 2 mM Ca^{2+}) was incubated with different enzymes for 30 min at 37°; BSA (1 mg in 0.1 ml) was then added, as well as (^{3}H-MeSO$_2$)$_2$TCB to a final concentration of 15 nM. The samples were incubated for another 30 min at 37° and were then assayed as described in Materials and Methods. For the control cytosol, the specific binding was 0.57 ± 0.03 pmol = 100%. The effect of enzymes is expressed as percentage of control specific binding remaining after treatment. Each value represents the mean ± SD of three experiments.

Treatment	Dose	Specific binding
	μg	%
Control	0	100
Pronase	250	46 ± 6
	500	17 ± 9
Papain	500	62 ± 4
Trypsin	100	167 ± 11
	250	161 ± 8
	500	149 ± 13
DNase	500	98 ± 4
RNase	500	102 ± 7

pmol/mg of cytosolic protein and a K_d of 13 nM. Calculations from data obtained by several saturation experiments at different temperatures constantly gave B_{max} values in the range of 30–70 pmol/mg of protein and K_d values ranging between 2.5 and 15.0 nM. These data thus indicate a finite number of binding sites with a high affinity for (^{3}H-MeSO$_2$)$_2$TCB in rat lung cytosol.

The reproducibility between assays was regarded as acceptable and might be exemplified by the saturation analysis at 0–4° on one batch of cytosol that gave a B_{max} = 53 ± 5 pmol/mg of cytosolic protein and a K_d = 9.4 ± 3.7 nM (n = 5).[3] The cytosol was stored in a concentrated form and diluted for each experiment, and differences in protein concentration occurred [as determined by the Lowry method (14)]. The B_{max} was independent of such differences in protein concentration whereas the K_d varied to a greater extent depending on protein concentration.

Tissue distribution. The tissue distribution of specific (^{3}H-MeSO$_2$)$_2$TCB binding was investigated. Even considering the underestimation of binding sites due to subsaturating conditions [2 mg of cytosolic protein, 10 nM (^{3}H-MeSO$_2$)$_2$TCB], the lung cytosol contained by far the largest number of binding sites, 4 pmol/mg of protein. Under identical conditions, detectable but significantly lower amounts were found in the prostate (0.17 pmol/mg of protein), the kidney (0.14 pmol/mg of protein), and the large intestine (0.06 pmol/mg of protein). When the binding in the different tissues was studied at different temperatures and timepoints, no differences in binding due to differences in kinetics were seen (data not shown). No other tissues examined contained detectable amounts of binding.

Effects of hydrolytic enzymes on the specific (^{3}H-MeSO$_2$)$_2$TCB binding. Table 1 summarizes the effects of various enzymes on specific (^{3}H-MeSO$_2$)$_2$TCB binding. The most dramatic effect was seen with pronase, which reduced the specific binding to 17% of the control value. Papain reduced the specific binding to 62% whereas trypsin increased the specific binding to 167% of the control value. Possibly, trypsin makes the binder more accessible to the ligand. Neither RNase nor DNase treatment had any effects. These data are compatible with a proteinaceous nature of the specific (^{3}H-MeSO$_2$)$_2$TCB binder. However, this lung cytosolic protein appears to be relatively stable to proteolysis, since even at high concentrations of protease significant amounts of specific binding remained.

Gel permeation chromatography and density gradient centrifugation. In order to further characterize the phys-

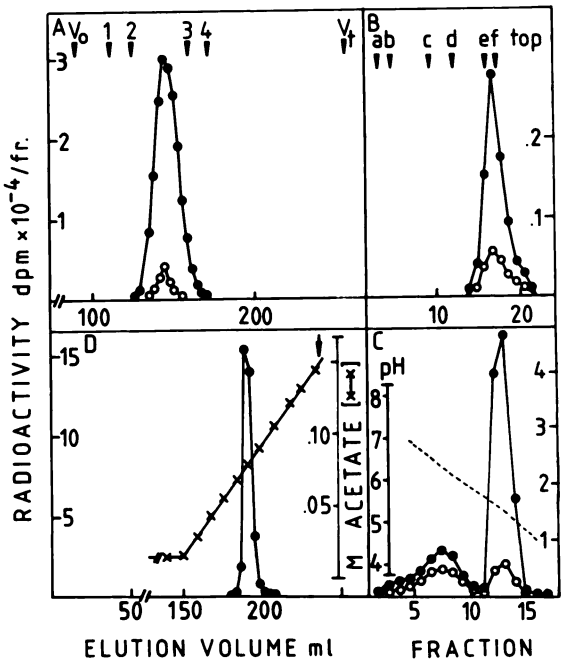

FIG. 6. *Physicochemical characterization of (^{3}H-MeSO$_2$)$_2$TCB-labeled lung cytosol*

Rat lung cytosol (2–5 mg of protein/ml) in buffer A was incubated with 10–20 nM (^{3}H-MeSO$_2$)$_2$TCB at 37° for 1 hr in the presence (○) or absence (●) of a 100-fold molar excess of unlabeled (MeSO$_2$)$_2$TCB and then placed on ice for 15 min before further analysis. A, labeled cytosol (4 ml) was applied to Sephadex G-75 columns (2.6 × 65 cm) equilibrated in buffer A and eluted with the same buffer at a flow rate of 1.9 ml/cm^2/hr. Four-ml fractions were collected and assayed for radioactivity. The columns were calibrated with Blue Dextran 2000 (V_o), [^{14}C]glucose (V_t), and the following standard proteins: *1*, ovalbumin (28.6 Å); *2*, DNase I (24.6 Å); *3*, myoglobin (20.1 Å); and *4*, cytochrome *c* (17.9 Å) (20). B, labeled cytosol (0.2 ml) was layered onto linear 5–20% (w/v) sucrose density gradients and then centrifuged, fractionated, and assayed as described in Materials and Methods. ^{14}C-labeled standard proteins were run concomitantly: *a*, aldolase (7.9 S); *b*, IgG (6.6 S); *c*, BSA (4.4 S); *d*, ovalbumin (3.5 S); *e*, myoglobin (2.0 S); and *f*, cytochrome *c* (1.7 S) (20). C, labeled cytosol (0.3 ml) was subjected to isoelectric focusing as described in Materials and Methods. Each fraction represents a 3-mm gel slice. The pH gradient is marked by the dashed line. D, labeled cytosol (6 ml) was applied to a CM-Sepharose column (2.6 × 8.0 cm) and chromatographed as described in Materials and Methods. The arrow indicates the start of a 1 M acetate wash to elute material still bound after the gradient elution. In all analysis, 80–100% of the specific binding loaded is accounted for by the isolated peak fractions. Only fractions containing radioactivity above background levels have been plotted.

[3] Values represent the mean ± SD. Numbers in parentheses indicate the number of experiments.

icochemical nature of the (^{3}H-$MeSO_2)_2$TCB-binding protein, the complex was subjected to gel permeation chromatography on a Sephadex G-75 column. This resulted in the inclusion of a single (^{3}H-$MeSO_2)_2$TCB-binding entity with an elution volume (V_e) of 144 ± 0.8 ml (n = 4)[3] (Fig. 6A). This peak was almost completely abolished in the presence of a 100-fold molar excess of unlabeled $(MeSO_2)_2$TCB. Calibration of the column with standard proteins and correlations of the distribution coefficients, K_d, with the known Stokes radii for the proteins described by Sherman *et al.* (20) gave a Stokes radius for the saturable (^{3}H-$MeSO_2)_2$TCB-binding protein of 22 ± 0.2 Å (n = 4).[3]

When aliquots of the same (^{3}H-$MeSO_2)_2$TCB-labeled rat lung cytosol were run on 5–20% (w/v) linear sucrose gradients, a single radioactive peak sedimented with an apparent sedimentation coefficient of 1.7 ± 0.2 S (n = 6),[3] as determined by concomitant sedimentation of ^{14}C-labeled marker proteins (Fig. 6B). This peak was significantly reduced by a 100-fold molar excess of unlabeled $(MeSO_2)_2$TCB. A 100-fold excess of $(MeSO_2)_2$TCB was not sufficient to saturate the specific binding sites at the protein concentration (2 mg/ml) used and this could explain why the radioactive peaks were not completely abolished.

By use of S and R_s values, it was possible to calculate certain physical properties of the (^{3}H-$MeSO_2)_2$TCB-binding protein. It was assumed that the partial specific volume of the macromolecule was 0.725 cm^3/g and that the solvation factor was 0.2 g of solvent/g of solute, both of which are typical values for proteins (21). The molecular weight was calculated according to the method of Siegel and Monty (18): $M_r = 424SR_s$. This gave an apparent molecular weight of 16,000. The equation (22) $f/f_0 = 1.393(R_s/M_r^{1/3})$ gave a frictional ratio of 1.22. Assuming that the protein had a shape of a prolate ellipsoid, an axial ratio of 4 was obtained from a published table (23).

Determination of isoelectric point. When subjected to isoelectric focusing in polyacrylamide gels the (^{3}H-$MeSO_2)_2$TCB-labeled lung protein focused as one major radioactive peak at pH 5.3 ± 0.1 (n = 4)[3] (Fig. 6C). This peak was almost completely abolished by a 100-fold molar excess of unlabeled $(MeSO_2)_2$TCB, in contrast to the smaller and broader radioactive peak which focused at a more basic pH and which was only partially abolished by an excess of unlabeled $(MeSO_2)_2$TCB. In line with these results, chromatofocusing of (^{3}H-$MeSO_2)_2$TCB-labeled rat lung cytosol consistently gave an elution pattern with a single, displaceable specific peak eluting at pH 5.2–5.4 (data not shown).

Cation exchange chromatography. When (^{3}H-$MeSO_2)_2$TCB-labeled rat lung cytosol in buffer B was applied to a CM-Sepharose column and eluted with a linear acetate and pH gradient, the specific binding protein eluted as a single radioactive peak at an acetate concentration of 75 mM (Fig. 6D). The pH in the peak fraction was 4.9, i.e., slightly lower than the pI obtained by isoelectric focusing or chromatofocusing. The protein profile, as determined by the absorbance at 280 nm, showed that the major protein peaks did not correspond to the radioactivity and this method therefore appears promising for the purification of the binding protein.

Competition studies. As shown in Fig. 7, all PCB methyl sulfones tested competed with the (^{3}H-$MeSO_2)_2$TCB binding. As expected, approximately 15 nM of $(MeSO_2)_2$TCB was required to displace 50% of the specific (^{3}H-$MeSO_2)_2$TCB binding. 4-Methylsulfonyl-2,2′,4′,5,5′-pentachlorobiphenyl competed even more effectively than the corresponding unlabeled $(MeSO_2)_2$TCB with an approximate IC_{50} value of 4 nM, whereas 4-methylsulfonyl-2,3′,4′,5-tetrachlorobiphenyl and 4-methylsulfonyl-2,2′,5,5′-tetrachlorobiphenyl were weaker competitors with approximate IC_{50} values of 50 and 65 nM, respectively. None of the PCB tested competed with (^{3}H-$MeSO_2)_2$TCB binding, except 2,2′,4,4′,5,5′-hexachlorobiphenyl which had an approximate IC_{50} value of 3 μM. It was thus about 100 to 1000 times less potent as a competitor than the PCB methyl sulfones.

Other compounds were tested for their capacity to compete with (^{3}H-$MeSO_2)_2$TCB binding. First, some halogenated and nonhalogenated aromatic hydrocarbons were tested. Neither 2,3,7,8-tetrachlorodibenzofuran, 3-methylcholanthrene, nor benzo(a)pyrene competed with specific (^{3}H-$MeSO_2)_2$TCB binding in rat lung cytosol at the concentrations tested. Due to the limited solubility of these compounds, the highest concentration tested only represented a 200-fold molar excess as compared to (^{3}H-$MeSO_2)_2$TCB. Accordingly, competition below the 0.5% level could not be assessed. Two additional arylmethyl sulfones, methylsulfonyl-pentachlorobenzene and 3-methylsulfonyl-2,2′-bis(4-chlorophenyl)-1,1-dichloroethylene, were tested but neither one of these competed for (^{3}H-$MeSO_2)_2$TCB binding. This indicates that the presence of a methylsulfonyl moiety is not the only requirement for a high affinity to the macromolecule in lung cytosol.

Finally, some common steroids were tested for binding. Only the progesterone derivatives competed for the (^{3}H-$MeSO_2)_2$TCB binding, albeit with much lower affinity than the methyl sulfone metabolites of PCB. Progesterone had an approximate IC_{50} value of 6 μM, whereas 5α-pregnane-3,20-dione and 5β-pregnane-3,20-dione had IC_{50} values of 1.5 and 9 μM, respectively. None of the glucocorticoids (corticosterone, dexamethasone), mineralocorticoids (aldosterone), estrogens (17β-estradiol, estrone, diethylstilbestrol), or androgens (testosterone, 5α-dihydrotestosterone) tested exhibited any affinity for the (^{3}H-$MeSO_2)_2$TCB-binding protein in lung.

(^{3}H-$MeSO_2)_2$TCB binding to mouse lung cytosol and to a protein purified from rabbit uterus. Preparations of lung cytosol from C57BL/6J mice were also found to contain specific (^{3}H-$MeSO_2)_2$TCB-binding sites. As shown in Table 2, no significant physicochemical differences could be demonstrated when this binding entity was compared to the specific (^{3}H-$MeSO_2)_2$TCB-binding protein in rat lung cytosol.

In view of the physicochemical characteristics of the (^{3}H-$MeSO_2)_2$TCB-binding protein in rat lung and the observed competition by progesterone for specific (^{3}H-$MeSO_2)_2$TCB binding in lung cytosol, it was of interest

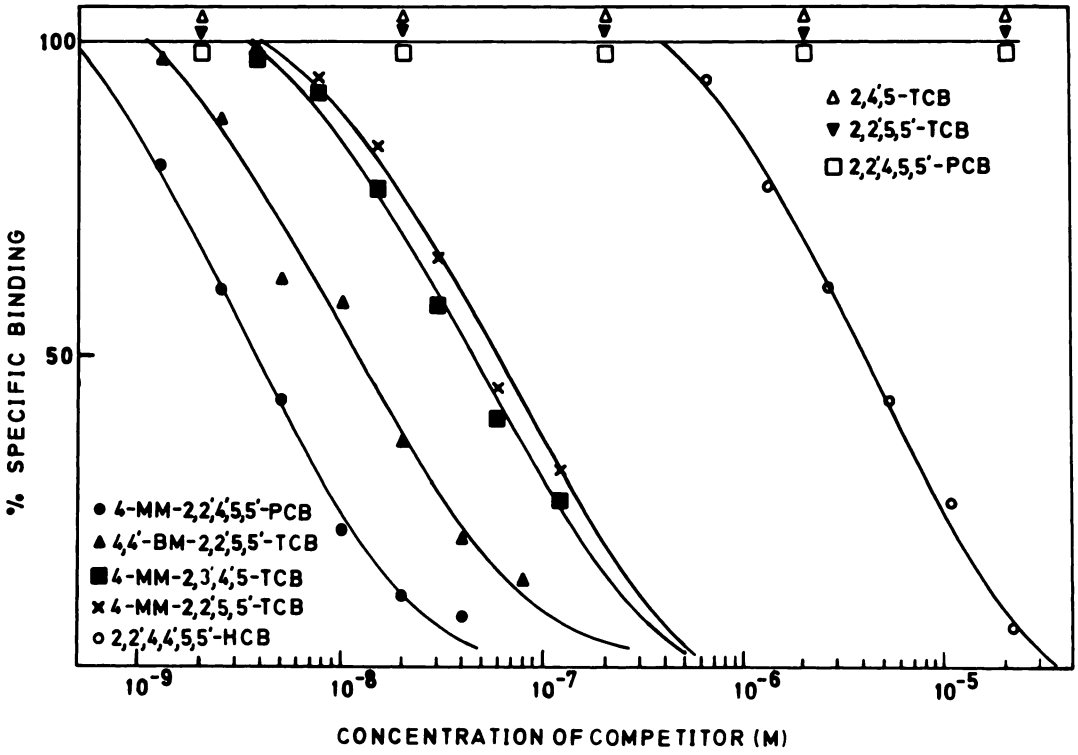

FIG. 7. *Competition of PCB and metabolites of PCB with the specific binding of (^{3}H-MeSO$_2$)$_2$TCB to lung cytosol*

The specific binding of (^{3}H-MeSO$_2$)$_2$TCB was measured as described in Materials and Methods. The concentration of (^{3}H-MeSO$_2$)$_2$TCB was 15 nM and the concentration of the unlabeled congener is given on the abscissa. Each value represents the mean of triplicate determinations in two separate experiments.

to test the binding of (^{3}H-MeSO$_2$)$_2$TCB to a similar low molecular weight protein purified from rabbit uterus, uteroglobin, known to bind progesterone (cf. Discussion). When uteroglobin was incubated with (^{3}H-MeSO$_2$)$_2$TCB and then analyzed by gel permeation chromatography on Sephadex G-75 and by sucrose gradient centrifugation, one single radioactive peak was observed which was completely abolished when the incubations were performed in the presence of a 100-fold molar excess of unlabeled (MeSO$_2$)$_2$TCB (data not shown).

Table 2 summarizes data obtained in the present study and data collected from the literature. As can be seen, the (^{3}H-MeSO$_2$)$_2$TCB-binding protein in rat lung, and uteroglobin in rabbit uterus and lung show similarities with regard to (a) physicochemical characteristics. The two proteins have similar apparent molecular weights [although (^{3}H-MeSO$_2$)$_2$TCB-labeled uteroglobin had a slightly smaller Stokes radius and a slightly lower S value than the rat (^{3}H-MeSO$_2$)$_2$TCB-binding protein], similar isoelectric points, and identical behavior on CM-Sepharose. (b) In regard to binding characteristics, both proteins bind (^{3}H-MeSO$_2$)$_2$TCB, and both proteins show similar affinities for progesterone (K_d = 10^{-6}–10^{-7} M). (c) Both proteins show similar selective tissue distribution patterns. A more complete comparison of the proteins will have to await the purification of the rat lung protein.

DISCUSSION

(MeSO$_2$)$_2$TCB and several other PCB methyl sulfones have previously been shown to be selectively accumulated in the mucosa of the respiratory tract of mice (7, 24, 25). The accumulated sulfones are generally formed from PCB molecules with specific chlorination patterns (i.e., chlorine atoms in positions 2, 4′, and 5 and vicinal hydrogen atoms in positions 3 and 4), and the position of the sulfone moiety is also of significance for the tissue affinity (7, 24, 25). Although 3- and 4-methylsulfonyl-PCBs seem to be formed in about equal amounts, the 4-methylsulfonyl-PCBs are always the major isomers in the lung.

In the present paper, microautoradiography was used to demonstrate that the uptake of (^{3}H-MeSO$_2$)$_2$TCB in the respiratory tract in rats is due to a selective binding in the Clara cells. Furthermore, *in vitro* studies show that cytosol from lungs of Sprague-Dawley rats and C57BL/6J mice contains saturable, high affinity sites that bind (^{3}H-MeSO$_2$)$_2$TCB reversibly. Lower levels of specific binding sites also seem to be present in cytosolic preparations from prostate, kidney, and large intestine. Biochemical characterization suggests that the (^{3}H-MeSO$_2$)$_2$TCB-binding entity is an acidic protein (pI 5.3) with an apparent molecular weight of 16,000. Competition studies indicate that at least certain PCB methyl sulfones have similar affinities for the specific binding sites as (MeSO$_2$)$_2$TCB, whereas methyl sulfone derivatives of some other aromatic hydrocarbons do not compete.

It might be noted that all the PCB methyl sulfones tested are known to accumulate in the lung bronchial mucosa of mice according to whole-body autoradiography

TABLE 2

Comparison of (^{3}H-MeSO$_2$)$_2$TCB-binding proteins in rat and mouse lung cytosol with uteroglobin

Physicochemical characteristics and binding properties of rat and mouse (^{3}H-MeSO$_2$)$_2$TCB-binding proteins and uteroglobin were determined as described in Materials and Methods or obtained from the literature (indicated by parentheses referenced to footnotes). Experimental values represent mean ± SD of four to six experiments.

Parameter	Specific (^{3}H-MeSO$_2$)$_2$TCB binder		Uteroglobin from rabbit
	Rat	Mouse	
S value	1.7 ± 0.2	1.6 ± 0.2	1.5 ± 0.3 (1.56)[a]
R_s	22.0 ± 0.2	22.1 ± 0.3	20.9 ± 0.3 (18.4)[a]
M_r	15,800	15,000	13,300 (14,000–16,000)[a]
pI	5.3 ± 0.1	ND[b]	(5.4)[c]
CM-Sepharose[d]	75 mM	ND	(75 mM)[e]
Affinity for			
(^{3}H-MeSO$_2$)$_2$TCB	Yes	Yes	Yes
Progesterone	Yes	ND	(Yes)[f]
Tissue distribution (male animals)	Lung > > prostate > kidney	ND	(Lung >> epididymis > kidney)[g]

[a] Nieto *et al.* (28).
[b] ND, not determined.
[c] McGaughey *et al.* (35).
[d] Concentration of acetate required for elution.
[e] Torkkeli *et al.* (29).
[f] Beato (36).
[g] Torkkeli *et al.* (37).

(7, 24, 25). No PCB methyl sulfones deviating from the proposed structural requirements for lung bronchial accumulation were available for testing. Among the four PCBs tested, only one, 2,2′,4,4′,5,5′-hexachlorobiphenyl, competes for specific (^{3}H-MeSO$_2$)$_2$TCB binding, although with at least a 100-fold lower affinity. This PCB is also known to accumulate as the unmetabolized compound in the lung bronchial mucosa of mice (7). This indicates that the methylsulfonyl group is not an absolute prerequisite for binding of PCB, but that its presence increases the affinity of the ligand by several orders of magnitude. Two aryl methyl sulfones were found to be incapable of displacing (^{3}H-MeSO$_2$)$_2$TCB from its specific binding sites in lung cytosol. These results indicate that the presence of a methylsulfonyl group as such is not sufficient for compounds to display an affinity for the (^{3}H-MeSO$_2$)$_2$TCB-binding protein. Interestingly, progesterone and some of its derivatives showed some affinity for the specific (^{3}H-MeSO$_2$)$_2$TCB-binding sites. Although the affinities were several orders of magnitude lower than for the PCB methyl sulfones, it cannot be ruled out that progesterone and/or metabolites thereof might constitute endogenous ligands for the lung protein.

There seems to be a relationship between uptake *in vivo* of (^{3}H-MeSO$_2$)$_2$TCB in rat lung and the presence in this tissue of specific (^{3}H-MeSO$_2$)$_2$TCB-binding sites. The structural requirements for *in vivo* accumulation of PCB methyl sulfones (7, 24, 25) seem to be valid also for their competition *in vitro* for specific (^{3}H-MeSO$_2$)$_2$TCB-binding sites in lung cytosol. It therefore seems reasonable to assume that the presence of an acidic, low molecular weight protein that recognizes and binds (^{3}H-MeSO$_2$)$_2$TCB is responsible for the selective tissue and cellular accumulation of PCB methyl sulfones seen *in vivo*. However, any conclusions as to the significance of these binding sites for the pulmotoxic symptoms observed following exposure to PCB (26) cannot be drawn at the present time. In order to gain further knowledge about this issue, studies on the possible effects of (MeSO$_2$)$_2$TCB on different pulmonary parameters are required.

As to the possible physiological role of the (^{3}H-MeSO$_2$)$_2$TCB-binding protein, some of the data in the present report may be of significance. First, progesterone and some progesterone derivatives compete for specific (^{3}H-MeSO$_2$)$_2$TCB binding. Interestingly, a heat- and acid-stable progesterone-binding protein has previously been described in rat lung (27). The reported concentration of progesterone-binding sites in rat lung cytosol (30–40 pmol/mg of protein) is compatible with the concentration of specific (^{3}H-MeSO$_2$)$_2$TCB-binding sites in lung cytosol found in the present study. The lung progesterone binder had a moderately high affinity ($K_d \sim 0.1$ μM) for [^{3}H]R5020 and appeared to be of low molecular weight. The authors hypothesized that the protein might serve to concentrate progesterone locally or to prevent binding of progesterone to the glucocorticoid receptor.

Second, the affinity of the (^{3}H-MeSO$_2$)$_2$TCB binder for progesterone as well as its physicochemical characteristics (S value, R_s, M_r, and pI) agree well with the properties of a protein purified from rabbit uterus and lung called uteroglobin or blastokinin (28, 29). Although it has been suggested that this protein, which is positively regulated by progesterone, might act as a stimulatory factor on the blastocyst, as a carrier of progesterone, or as an agent protecting the blastocyst from an excess of progesterone, the physiological role of uteroglobin is still unclear (for a review, see Ref. 30). In order to investigate a possible relationship between the uteroglobin-like (^{3}H-MeSO$_2$)$_2$TCB binder in rat lung and the (^{3}H-MeSO$_2$)$_2$TCB-binding uteroglobin in rabbit uterus and lung, it is necessary to purify the (^{3}H-MeSO$_2$)$_2$TCB-binding protein from rat lung.

It will also be of interest to investigate if the synthesis of a uteroglobin-like (^{3}H-MeSO$_2$)$_2$TCB-binding protein can be hormonally induced in the rat uterus, as PCB methyl sulfones have been shown to accumulate in the uterine secretion of pregnant mice (31). Since PCB have been claimed to be both pulmotoxic (26) and to cause reproductive disturbances (32), it is intriguing that PCB methyl sulfones interact with high affinity with a uteroglobin-like protein present in rat lung and possibly in rat uterus. It cannot be excluded that these methyl sulfones elicit their toxic effects by displacing an endogenous ligand from its binding sites on the protein, thereby preventing this protein from fulfilling some important biological function.

A recent paper (33) has proposed an evolutionary relationship between rabbit uteroglobin and rat prostatic steroid-binding protein (see Ref. 18 in Ref. 33) based on computer analysis of the amino acid sequences. This is of particular interest for several reasons. First, prostatic steroid-binding protein also binds progesterone (see Ref. 32 in Ref. 33). Second, uteroglobin-like antigens have been shown in seminal fluid (see Ref. 35 in Ref. 33). Third, polycyclic aromatic hydrocarbons have been

shown to interact with the prostatic secretions (34). If there exists a relationship not only between the rat (^{3}H-MeSO$_2$)$_2$TCB-binding protein and rabbit uteroglobin, but also between these proteins and rat prostatic steroid-binding protein, it is possible that the specific (^{3}H-MeSO$_2$)$_2$TCB binding found in the prostate represents binding to the prostatic steroid-binding protein. Clearly, further work is needed to investigate these low molecular weight steroid-binding proteins, both with regard to their role in targeting of exogenous toxic compounds and to their physiological functions.

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REFERENCES

1. Kimbrough, R. D. (ed.). *Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products.* New York, Elsevier/North-Holland, (1980).
2. Poland, A., and J. C. Knutson. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. *Annu. Rev. Pharmacol. Toxicol.* **22**:517–554 (1982).
3. Mizutumi, T., K. Yamamoto, and K. Tajina. Sulfur-containing metabolites of chlorobiphenyl isomers, a comparative study. *J. Agric. Food Chem.* **26**:862–866 (1978).
4. Bakke, J. E., V. J. Feil, and Å. Bergman. Metabolites of 2,4′,5-trichlorobiphenyl in rats. *Xenobiotica* **13**:555–564 (1983).
5. Preston, B. D., J. A. Miller, and E. C. Miller. Reactions of 2,2′,5,5′-tetrachlorobiphenyl 3,4-oxide with methionine, cysteine and glutathione in relation to the formation of methylthio-metabolites of 2,2′,5,5′-tetrachlorobiphenyl in the rat and mouse. *Chem.-Biol. Interact.* **50**:289–312 (1984).
6. Brandt, I., E. Klasson-Wehler, J. Rafter, and Å. Bergman. Metabolism of 2,4′,5-trichlorobiphenyl: tissue concentrations of methylsulphonyl-2,4′,5-trichlorobiphenyl in germfree and conventional mice. *Toxicol. Lett.* **12**:273–278 (1982).
7. Bergman, Å., I. Brandt, and B. Jansson. Accumulation of methylsulphonyl derivatives of some bronchial seeking polychlorinated biphenyls in the respiratory tract of mice. *Toxicol. Appl. Pharmacol.* **48**:213–220 (1979).
8. Higuchi, K. (ed.). *PCB Poisoning and Pollution.* Tokyo, Kodansha Ltd., (1976).
9. Urabe, H., H. Koda, and M. Asahi. Present state of Yusho patients. *Ann. N. Y. Acad. Sci.* **320**:273–276 (1979).
10. Klasson-Wehler, E., Å. L. Bergman, and C. A. Wachtmeister. Synthesis of 4,4′-bis([^{3}H]methylsulphonyl)-2,2′,5,5′-tetrachlorobiphenyl. *J. Label. Cmpd. Radiopharm.* **20**:1407–1412 (1983).
11. Cadogan, J. I. G. A convenient new method for aromatic arylation. *J. Chem. Soc.* 4257–4258 (1962).
12. Moron, M., G. Sundström, and C. A. Wachtmeister. Polychlorinated biphenyls. VI. 2,3,7,8-Tetrachlorodibenzofuran, a critical byproduct in the synthesis of 2,2′,4,4′,5,5′-hexachlorobiphenyl by the Ullman reaction. *Acta Chem. Scand.* **27**:3121–3122 (1973).
13. Bergman, Å., and C. A. Wachtmeister. Synthesis of methanesulfonyl derivatives of 2,2,-bis(4-chlorophenyl)-1,1-dichloroethylene (p,p′-DDE), present in seal from the Baltic. *Acta Chem. Scand. B* **31**:90–91 (1977).
14. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. Protein measurement with Folin phenol reagent. *J. Biol. Chem.* **193**:265–275 (1951).
15. Dahlberg, E., M. Snochowski, and J.-Å. Gustafsson. Regulation of the androgen and glucocorticoid receptors in rat and mouse skeletal muscle cytosol. *Endocrinology* **108**:1431–1440 (1981).
16. Scatchard, G. The attraction of proteins for small molecules and ions. *Ann. N. Y. Acad. Sci.* **51**:660–672 (1949).
17. Porath, J. Some recently developed fractionation procedures and their application to peptide and protein hormones. *J. Appl. Chem.* **6**:233–244 (1963).
18. Siegel, L. M., and K. J. Monty. Determinations of molecular weights and frictional ratios of proteins in impure systems by use of gel filtration and density gradient centrifugation: application to crude preparations of sulfite and hydroxylamine reductase. *Biochim. Biophys. Acta* **112**:346–362 (1966).
19. Rice, R. H., and G. E. Means. Radioactive labeling of proteins *in vitro*. *J. Biol. Chem.* **246**:831–832 (1971).
20. Sherman, M. R., F. B. Tuazon, and L. K. Miller. Estrogen receptor cleavage and plasminogen activation by enzymes in human breast tumor cytosol. *Endocrinology* **106**:1715–1727 (1980).
21. Tanford, L. *Physical Chemistry of Macromolecules.* New York, John Wiley and Sons, 34 (1961).
22. Sherman, M. R. Physical-chemical analysis of steroid hormone receptors. *Methods Enzymol.* **36**:211–234 (1975).
23. Schachman, H. K. *Ultracentrifugation in Biochemistry.* New York, Academic Press, 239 (1959).
24. Bergman, Å., I. Brandt, P. O. Darnerud, and C. A. Wachtmeister. Metabolism of 2,2′,5,5′-tetrachlorobiphenyl: formation of mono- and bis-methyl sulphone metabolites with a selective affinity for the lung and kidney tissues in mice. *Xenobiotica* **12**:1–7 (1982).
25. Brandt, I., and Å. Bergman. Bronchial mucosal and kidney cortex affinity of 4- and 4,4′-substituted sulphur-containing derivatives of 2,2′,5,5′-tetrachlorobiphenyl in mice. *Chem.-Biol. Interact.* **34**:47–55 (1981).
26. Shigematsu, N., S. Ishimaru, R. Saito, T. Ikedu, K. Matsuba, K. Suginama, and Y. Masuda. Respiratory involvement in polychlorinated biphenyls poisoning. *Environ. Res.* **16**:92–100 (1978).
27. Moser, E. H., and G. Daxenbichler. Detection of a heat- and acid-stable 'progesterone'-binding protein in rat lung. *FEBS Lett.* **150**:347–353 (1982).
28. Nieto, A., H. Ponstingl, and M. Beato. Purification and quaternary structure of the hormonally induced protein uteroglobin. *Arch. Biochem. Biophys.* **180**:82–92 (1977).
29. Torkkeli, T., T. Krusius, and O. Jänne. Uterine and lung uteroglobins in the rabbit: two similar proteins with different hormonal regulation. *Biochim. Biophys. Acta* **544**:578–592 (1978).
30. Savouret, J.-F., and E. Milgrom. Uteroglobin: a model for the study of progesterone action in mammals. *DNA* **2**:99–104 (1983).
31. Brandt, I., P. O. Darnerud, Å. Bergman, and Y. Larsson. Metabolism of 2,4′,5-trichlorobiphenyl: enrichment of hydroxylated and methyl sulphone metabolites in the uterine luminal fluid of pregnant mice. *Chem.-Biol. Interact.* **40**:45–56 (1982).
32. Allen, J. R., D. A. Barsotti, L. K. Lambrecht, and J. P. Van Miller. Reproductive effects of halogenated aromatic hydrocarbons on nonhuman primates. *Ann. N. Y. Acad. Sci.* **320**:419–425 (1979).
33. Baker, M. E. Amino acid sequence homology between rat prostatic steroid binding protein and rabbit uteroglobin. *Biochem. Biophys. Res. Commun.* **114**:325–330 (1983).
34. Haaparanta, T., H. Glaumann, and J.-Å. Gustafsson. Induction of cytochrome P-450 dependent reactions in the rat ventral prostate by β-naphthoflavone and 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Toxicology* **29**:61–75 (1983).
35. McGaughey, R. W., and F. A. Murray. Properties of blastokinin: amino acid composition, evidence for subunits, and estimation of isoelectric point. *Fertil. Steril.* **23**:399–404 (1972).
36. Beato, M. Binding of steroids to uteroglobin. *J. Steroid Biochem.* **7**:327–334 (1976).
37. Torkkeli, T. K., K. K. Kontula, and O. A. Jänne. Hormonal regulation of uterine blastokinin synthesis and occurence of blastokinin-like antigens in non-uterine tissues. *Mol. Cell. Endocrinol.* **9**:101–118 (1977).

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