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Effects of PBBs on Cattle. III. Target Organ Modification as Shown by Renal Function and Liver Biochemistry

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Efforts were made to more clearly delineate target organs and mechanisms of toxicity for PBBs in cattle. Methods were developed to obtain sequential liver biopsies on bovine heifers which yield 0.5 to 1.0 g of tissue. PBB was fed at a dose of 250 mg/head/day to Holstein heifers for 202 days. This dose produced no clinical signs of toxicity in any of the heifers, yet this produced tissue PBB concentration of greater than 100 times the FDA tolerance in body fat of 0.3 ppm. Liver biopsies (0.5–1.0 g each) were taken at days 0, 90, and 180. The liver tissue was homogenized and microsomes were prepared. Dithionite difference spectra were determined on the carbon monoxide treated microsome suspension and the cytochrome P-450 content determined. Also, the 100,000*g* supernatant was saved for ornithine decarboxylase analysis as a measure of hepatocyte proliferative activity. Results of the cytochrome P-450 analysis showed a significant ($p < 0.05$) two-fold elevation (per gram of wet liver) by day 90 and remained significantly ($p < 0.05$) elevated on day 180. The cytochrome P-450 values of control animals not receiving PBBs showed no such increase with time. The biopsy procedure appeared not to adversely affect the liver cytochrome P-450 concentration in the control heifers. These results show that PBBs at a dose of 250 mg/day induced the drug metabolism system of the liver, of which the cytochrome P-450 is a part, indicating that the liver is a potential target organ for PBBs. However, this has not been shown to cause clear signs of hepatotoxicity in the cow as determined from histopathology or serum enzyme analyses. The observed elevation of gross liver weights of the PBB-treated animals might be an expected consequence of the cytochrome P-450 induction. In contrast to rodents, the kidney has been identified by histopathology as a target organ for PBB toxicity in cattle. However, renal function studies with ^{131}I-sodium-iodohippurate and ^{125}I-sodium iodothalamate in PBB treated cows indicated that PBB toxicity to the kidney did not affect glomerular filtration rate or effective renal plasma flow even though nephrotoxic effects were produced. From these studies, both liver (as expected) and kidney (unexpected) were affected by PBBs. For liver this did not result in hepatotoxicity while for kidney nephrotoxicity was produced but could not be mechanistically explained.

Introduction

The continued controversy surrounding the effects and economic impact of chronic polybrominated biphenyl (PBB) exposure in dairy cattle is at least partially attributable to the virtual absence of information regarding target organ specificity, metabolism of xenobiotic compounds in cattle, and the potential ramifications to human health via the food chain.

After the impact of PBB contamination became apparent (*1*), feeding trials were conducted that established the PBB level required to elicit toxicity and provided clinicopathological definition of the toxicity syndrome (*2–4*). Histopathological studies (*3*) together with the clinical chemistry of the blood and urine of PBB treated dairy cattle (*4*), identified affected tissues and suggested target organs. Renal pathology was an outstanding consequence of acute PBB doses but was not evident at lower chronic doses. Histopathological alterations of liver were evident at acute PBB doses but were not as pronounced as the renal lesions (*4*). However, hepatic

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alteration was also evident at lower chronic doses of PBB (*2*). These results suggested that both liver and kidney were target organs for PBB toxicity.

Although liver has been described as a common target organ for other halogenated hydrocarbons (*5*), at the time these studies were initiated, there was only one report of hepatic response in PBB treated rats (*6*). During the course of these studies, subsequent reports also described the hepatotoxic effects of PBB in rats (*7–9*).

We initiated multifaceted studies aimed at identifying target organs for PBB toxicity and potential mechanisms of toxicity in dairy cattle. Furthermore, any identification of target organ responses could be related to previously determined clearance and distribution factors, histopathological changes, and tissue concentration of PBB in the dairy cow, relationships difficult to make in rats.

For assessment of hepatic involvement, a dose of 250 mg PBB/day was chosen as the optimum defined dose which appeared likely to elicit hepatic effects [liver weight was elevated by this dose (*2*)] without producing acute toxicosis having additional clinical effects (i.e., feed intake, nutrition, renal involvement, etc.) which would confound the interpretation of results (*2–4*). Hepatic microsome and cytochrome P-450 content were chosen as parameters indicative of specific hepatic response to xenobiotic compounds (*5*). Hepatic ornithine decarboxylase activity was chosen as a measure of post-injury cellular proliferation in the PBB treated liver (*10*). For assessment of renal effects, renal function (*11*) was determined at acute PBB doses known to elicit pathological lesions of the kidney (*3, 4, 11*). Serum protein patterns were assessed in cows and calves in an effort to detect both nonspecific and specific organ and tissue effects of PBB exposure. The results of these studies are summarized in this paper.

Materials and Methods

Specific reagents used in tissue preparations or assays were from the following sources: ethylenediaminetetraacetic acid disodium salt and sodium dithionite (J. T. Baker Co.); dithiothreitol and pyridoxal phosphate monohydrate (A grade, Calbiochem); aminooxyacetic acid hemihydrochloride, Grade II, semicarbazide hydrochloride, and L-ornithine hydrochloride, Sigma grade (Sigma Chemical Co.); DL-ornithine monohydrochloride (1-^{14}C) (49.9 mCi/mmole) (New England Nuclear). The PBB was a commercial mixture of PBB (FireMaster BP-6, lot 6244 A, Michigan Chemical Co.) containing predominantly hexabromobiphenyl but also containing substantial penta- and heptabromobiphenyls. All other general reagents used were the best obtainable grade.

Liver samples (0.5–1.0 g) were obtained by biopsy of dairy heifers before dosing and at 90 and 180 days after the start of the PBB dosing period. Experimental heifers (400 kg each) were dosed daily with 250 mg FireMaster BP-6 (lot 6244A) in gelatin capsules for 202 days. Finely ground FireMaster BP-6 was mixed with ground corn to prevent caking and administered orally in gelatin capsules. Control heifers matched in size and age, were biopsied at the same time and received no PBB.

Each liver biopsy specimen was immediately frozen at −70°C after biopsy and stored frozen (−20°C) until homogenized. An individual homogenate was prepared from 0.5–0.7 g of liver biopsy specimen after removing clots, connective tissue, and damaged tissue by teasing and washing with cold isotonic saline. All biopsies from an individual cow were homogenized on the same day.

Homogenates consisted of one part minced tissue (weight) plus four parts (volume) of cold homogenization buffer (0.25 *mM* KPO_4, pH 7.3 containing 5 *mM* Na EDTA and 10 *mM* dithionite) and were done in a Duall 23 conical glass tissue homogenizer with a motor-driven Teflon pestle (Kontes) immersed in ice. Five passes were made at medium motor speed. A 50 μl aliquot of the crude homogenate was saved for protein determination. The remaining crude homogenate was centrifuged at 10,000*g*, 2°C, for 25 min. The supernatant was then centrifuged at 100,000*g*, 2°C, for 60 min. The 100,000*g* supernatant was frozen until ornithine decarboxylase and protein assays could be done. The microsomal pellet from the 100,000*g* centrifugation was resuspended by gentle rehomogenization in 1.5 ml of cold 1.15% KCl and again centrifuged at 100,000*g*, 2°C, for 60 min. The washed microsomal pellet was again resuspended as before in 1.5 ml of 1.15% KCl and stored overnight at 2°C until cytochrome spectra could be run the next day.

Cytochrome P-450 was quantitated by dithionite difference spectra of carbon monoxide treated microsomes as described by Matsubara (*12*). Aliquots (0.1 and 0.15 ml) of microsome suspension approximately equivalent to that from 33 or 50 mg whole liver (ca 0.5–0.75 mg MIC protein/ml) were diluted to 1.0 ml with 1.15% KCl and bubbled for 2 min with carbon monoxide. After transfer to both sample and reference spectrophotometer cuvets, 5 mg dithionite was added to the sample cuvet and the difference spectrum determined after 1 min by scanning in a Beckman DB-GT double-beam spectrophotometer with recorder. For quantitation of cytochrome P-450, the molar extinction difference of 104 mM^{-1} cm^{-1} between 450 and 490 nm was used (*12*).

Ornithine decarboxylase activity was determined by measuring $^{14}CO_2$ liberated from D,L-(1-^{14}C)-ornithine. The assay, modified from Morley (*13, 14*), contained 0.2 *mM* pyridoxal phosphate, 25 *mM* potassium phosphate, pH 7.3, 2.5 *mM* dithiothreitol, 0.01 *mM* aminooxyacetate, and 150 or 300 μl of 100,000*g* supernatant preparation in 0.5 ml total assay volume. Aminooxyacetate inhibited nonspecific decarboxylation of ornithine (*15*). Reaction was initiated by the addition of 0.1 μCi of ^{14}C-ornithine (final assay concentration = 0.36 *mM*). Reactions were routinely incubated in septum stoppered culture tubes for 2 hr at 37°C after which the reaction was stopped by the addition with vortex mixing of 0.25 ml of 4*M* citric acid to liberate $^{14}CO_2$. An additional 1 hr incubation allowed complete release of $^{14}CO_2$. The released $^{14}CO_2$ was trapped in 0.3 ml of ethanolamine:methylcellosolve (1:2, v/v) contained in a polyethylene trap (Kontes) suspended from the rubber septum stopper. The entire trap and its contents were counted by liquid scintillation. Blank reactions complete with all assay components and enzyme preparation were incubated for the same time as the assays but contained 1.0 *mM* semicarbazide, an inhibitor of ornithine metabolism (*13*).

Under these conditions, the reaction was linear for at least 2 hr. Liver homogenate prepared from a thioacetamide-treated guinea pig (150 mg/kg) (*16*) in which ornithine decarboxylase was elevated approximately 400-fold over that of untreated liver served as a positive control with each set of assays.

The homogenization buffer used prevented loss of ornithine decarboxylase activity and at the same time was found to give identical cytochrome P-450 yields as 1.15% KCl (*12*). The washing of microsomes by resedimentation in 1.15% KCl was necessary in order to remove dithiothreitol which interferred with cytochrome P-450 spectral analysis. Hence with this single homogenization both ornithine decarboxylase and cytochrome P-450 could be quantitated. With this procedure microsome preparations were stable up to 2 days at 2°C while ornithine decarboxylase activity in the first 100,000*g* supernatant fluid was stable for months at −20°C.

Protein was quantitated by the Lowry method (*17*) with corrections for slight interference by dithiothreitol. Serum protein electrophoretic profiles were determined for all heifers of this experiment plus heifers of previous experiments in which the dose was 25 g PBB/day (*4*) and in calves from heifers given 250 mg PBB/day (*18*). Polyacrylamide slab gel electrophoresis of serum proteins used Isopore 5-20% acrylamide linear gradient precast gels (Isolab, Inc.) with 50 *mM* Tris adjusted to pH 9.00 with glycine as the tank buffer in an Ortec electrophoresis apparatus and pulsed power supply. Protein was visualized in the gel by staining with 0.2% Coomassie Brilliant Blue R-250 in glacial acetic acid:methanol:water (10:45:45), and diffusion destaining in the same solution without dye (Ortec Application Note AN 32).

Results

Clinical Toxicity

It should be stressed that during this experiment the feeding of PBB as FireMaster BP-6, 250/mg day for 202 days, to dairy heifers produced no clinical evidence of toxicity. Durst et al. (*2*) have also previously shown this to be a nontoxic dose in contrast to 25 g/day for 32 to 60 days which produced rapid and severe toxicosis (*2, 3*).

Liver Cytochrome P-450

The relative microsome content of liver biopsies is shown in Figure 1. There was an apparent relative increase in the weight of microsomal protein per weight of liver protein after treatment in the PBB-treated heifers, although the standard errors of the controls and of the PBB-treated heifers overlapped. This would suggest that the microsome content of the liver was increased by PBB treatment. The same pattern was seen when the absolute concentrations were compared rather than the relative changes as in Figure 1.

PBB treatment produced a clear elevation of the cytochrome P-450 content of liver microsomes as shown in Figure 2. There was at least a 50% increase in cytochrome P-450 content of liver microsomes in PBB-treated heifers by day 90. Further, the cytochrome content remained elevated through 180 days of treatment.

The elevation in liver cytochrome content in PBB treated heifers was even more pronounced when expressed on a per gram wet liver basis (Fig. 3), amounting to at least a twofold increase by day 90, but declining somewhat by day 180. This, together with results shown in Figure 2, indicate that the PBB treatment at 250 mg/day clearly induced cytochrome P-450 to increase in liver microsomes and that an even greater increase occurred in whole tissue. It seems likely that there was also a real elevation of total tissue microsome content, as suggested from Figure 1, in addition to a specific increase in the cytochrome P-450 content of those microsomes. This would account for the relative change in cytochrome P-450 content of whole tissue exceeding significantly the relative change in cytochrome

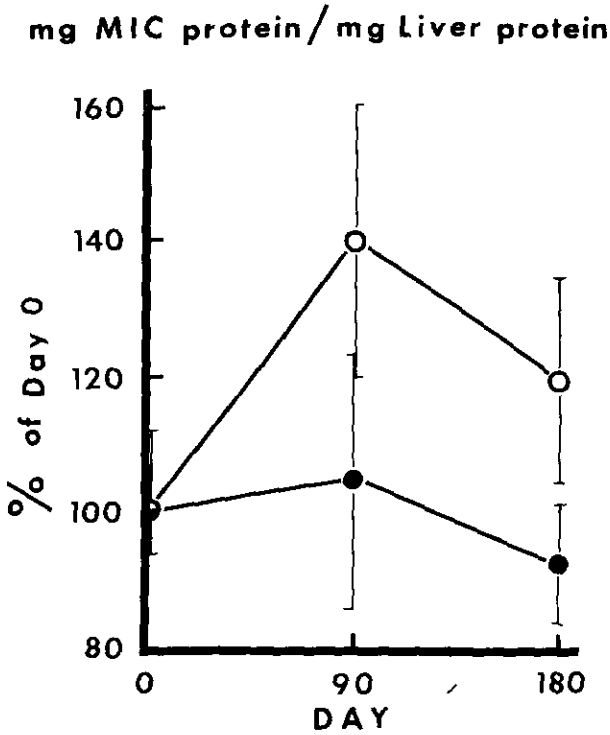

FIGURE 1. Relative change in liver microsome content of PBB treated and control heifers. Values shown are percent of the day zero (predosing) value per weight (mg) microsomal protein per weight (mg) liver protein for each group: (o) PBB-treated heifers, receiving 250 mg/day for 202 days; (•) controls received no PBB.

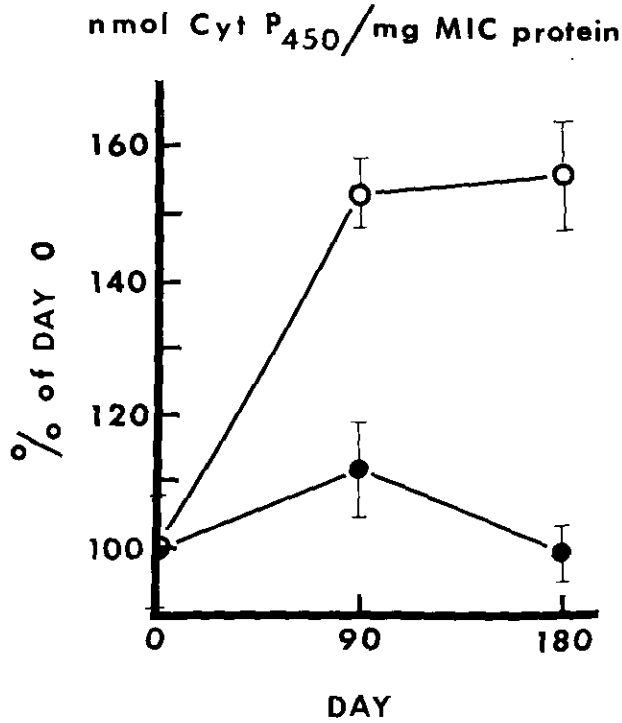

FIGURE 2. Relative change in cytochrome P-450 content of liver microsomes for PBB-treated and control heifers. Values shown are percent of the day zero (predosing) value for nmoles cytochrome P-450 per mg microsomal protein in (o) PBB-treated (250 mg/day) and (•) control heifers.

P-450 content of microsomes alone.

The ability of PBB to induce liver cytochrome P-450 is clearly illustrated in Figure 4, where the cytochrome P-450 content of liver microsomes is compared to the whole plasma PBB concentration of the treated heifers (*19*). The correlation coefficient was 0.91. Further, the basal values for microsomal cytochrome P-450 content are comparable to those found for unstimulated rat liver while the levels induced by PBB are comparable in terms of both relative increase and absolute concentration to those of rat liver after maximal induction with PBB (*7*) or phenobarbital (*12*).

Liver Ornithine Decarboxylase

While there was a clear elevation of hepatic cytochrome P-450 content by PBB, there was no such increase in hepatic ornithine decarboxylase activity. As shown in Figure 5, there was no detectable ornithine decarboxylase activity in either PBB-treated or control heifers at any time period examined. This was not due to assay insensitivity since basal ornithine decarboxylase activity in normal guinea pig liver was approximately 2 pmole $^{14}CO_2$/hr/mg liver protein with these same assay conditions. In our hands treatment of guinea pigs with thioacetamide (150 mg/kg IP) (*15*) resulted in a readily detectable 375-fold increase in liver ornithine decarboxylase activity to about 750 pmole CO_2hr/mg protein by 24 hr. Further, all ornithine decarboxylase assays done on bovine liver included, as a positive control, assays of thioacetamide treated guinea pig liver preparations. Activities obtained are shown in Figure 5 as an indication of what might be expected in the event of hepatic ornithine decarboxylase stimulation. It appears from this that the bovine liver has very low ornithine decarboxylase activity and it is clearly not significantly elevated by 250 mg PBB/day.

Renal Modification

The treatment of cows with high levels of PBB (25 g/day) was previously shown to produce unequivocal clinical toxicity (*2*) with characteristic histopathological lesions (*3*). Among the primary lesions observed was renal failure accompanied by extreme dilatation of collecting ducts and convoluted tubules with epithelial changes of hydropic

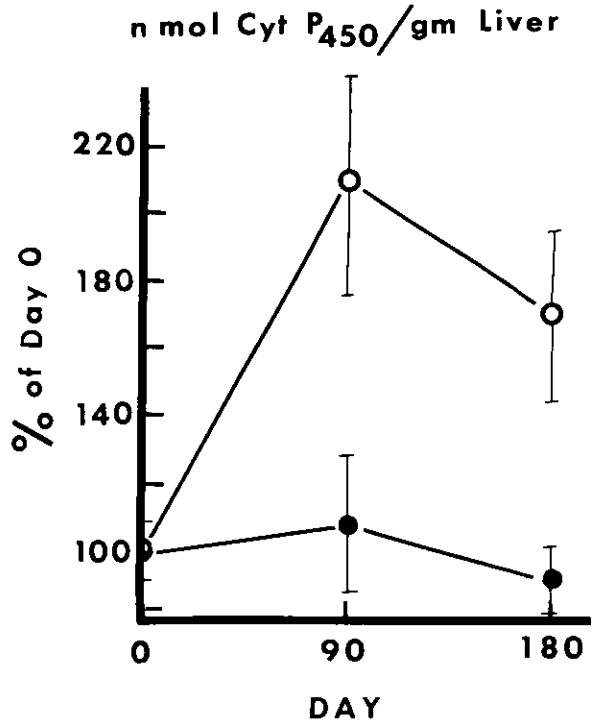

FIGURE 3. Relative change in cytochrome P-450 concentration of whole liver. Values shown are percent of the day zero (predosing) value for nmoles cytochrome P-450 per gram wet liver in (o) PBB-treated (250 mg/day) and (•) control heifers.

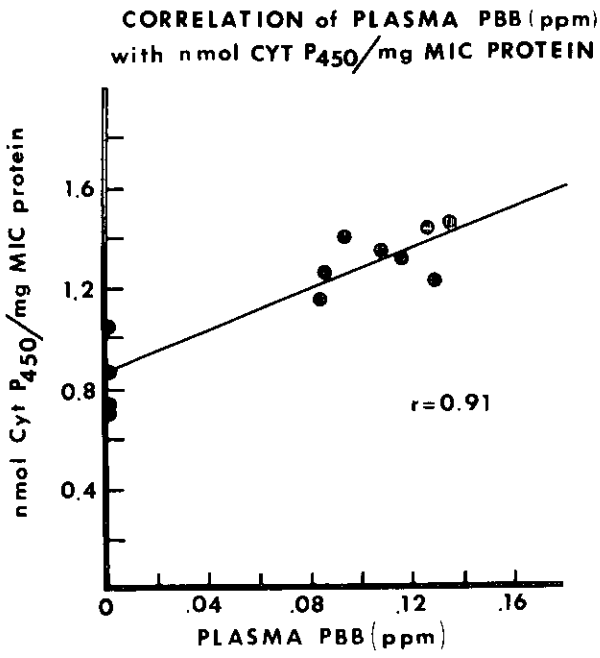

FIGURE 4. Correlation of plasma PBB concentration with hepatic microsomal cytochrome P-450 content in PBB-treated heifers. PBB was determined as previously described (*18*) in whole plasma from PBB-treated (250 mg/day) heifers at 0 (predosing), 90, or 180 days of dosing. Correlation coefficient $r = 0.91$.

degeneration and separation from the basement membrane (*3*). This would strongly suggest that renal function should be seriously impaired as a consequence of PBB toxicosis. However, recent efforts to determine the extent and site of such renal impairment through renal function studies during PBB toxicosis (*11*) gave no evidence of impairment.

Typical bovine renal function parameters are shown in Table 1 and are essentially the same for both PBB treated and control cows. After approximately 60 days, including 25 days of PBB treatment, the glomerular filtration rate and effective renal plasma flow remained normal (*11*). This indicates that in spite of the previously described renal lesions there is either (a) no impairment by PBB of either the glomerular filtration process or of the active transport processes or (b) the bovine kidney has virtually unlimited compensatory capacity in the event of nephron damage.

Serum Protein Changes

Upon electrophoretic analysis of serum protein in PBB treated heifers, there was no change in the protein pattern following treatment at doses of 250 mg/day or below for 60 days. In contrast, treatment with 25 g/day (*2*) caused significant changes in serum protein electrophoretic patterns. The changes are typified in Figure 6. Within 15 days after the first PBB dose was given, significant protein alterations were evident. Specifically the prealbumin band (the fastest migrating band) was markedly elevated on day 15 and increased thereafter. Conversely, serum albumin appeared to decrease somewhat on days 30 and 40. A band immediately below serum albumin did not stain with Coomassie Blue and hence was visualized by the "negative" staining characteristics. While this band has not been positively identified, its relative migration and staining characteristics suggest that it might be α_1-acid glycoprotein, an acute phase glycoprotein made by the liver during infection or febrile trauma, etc. (*20*). This band was markedly elevated on days 30, and 40. By day 30, SLDH and SGOT were also markedly elevated in this cow (*4*). There was also a slight increase in α-globulins (between transferrin and serum albumin) from days 15 through 40. Hence high PBB doses alter serum protein composition and it appears that those proteins of hepatic origin are primarily involved.

Newborn calves show dramatic changes in serum protein composition as a result of gut absorption of ingested colostrum immunoglobulins shortly after birth. A typical pattern is shown in Figure 7 for a newborn calf whose dam received 250 mg PBB/day for 60 days while pregnant. This pattern is indistin-

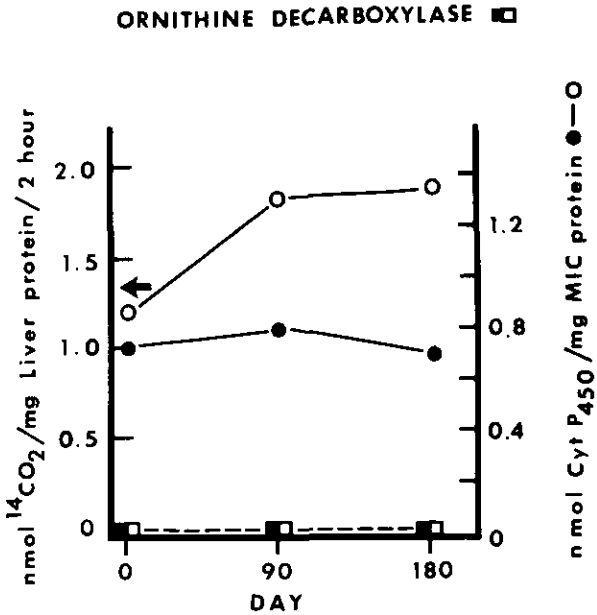

FIGURE 5. Failure of ornithine decarboxylase activities to increase in PBB-treated and control heifers. Microsomal cytochrome P-450 for (○) PBB-treated (250 mg/day) and (●) control heifers is shown for comparison. Ornithine decarboxylase activities for both (□) PBB-treated and (■) control heifers were not distinguishable from the reaction blanks in all samples tested. Activity of control assays with thioacetamide treated guinea pig liver is indicated by the arrow.

Table 1. Renal function parameters before and after PBB dosing in treated and control cows.[a]

Cow	Daily PBB dose, g[b]	Trial	Glomerular filtration rate, ml/kg/min	Effective renal plasma flow ml/kg/min
A	25	Predose	3.5	10.1
		Final	3.4	12.6
C	25	Predose	2.7	7.5
		Final	2.8	14.0
B	0	Predose	—	—
		Final	2.6	9.1
D	0	Predose	2.2	6.7
		Final	2.3	8.6

[a] Data are for initial and final of six renal function trials (days 0, 9, 15, 29, 49, 62–65).
[b] PBB dose given daily for 25 days. Predose trial is day 0 while final trial is 35 ± 1 days after the last dose for all cows.

guishable from that of a calf not receiving PBB. This calf showed a typical neonatal bovine serum protein pattern on day 1 prior to ingestion of colostrum. This pattern is characterized by the paucity of immunoglobulin in the serum. In contrast, by the second sampling period, the calf had ingested colostrum and transfer from the gut to blood had occurred. There appeared to be no impairment of gut

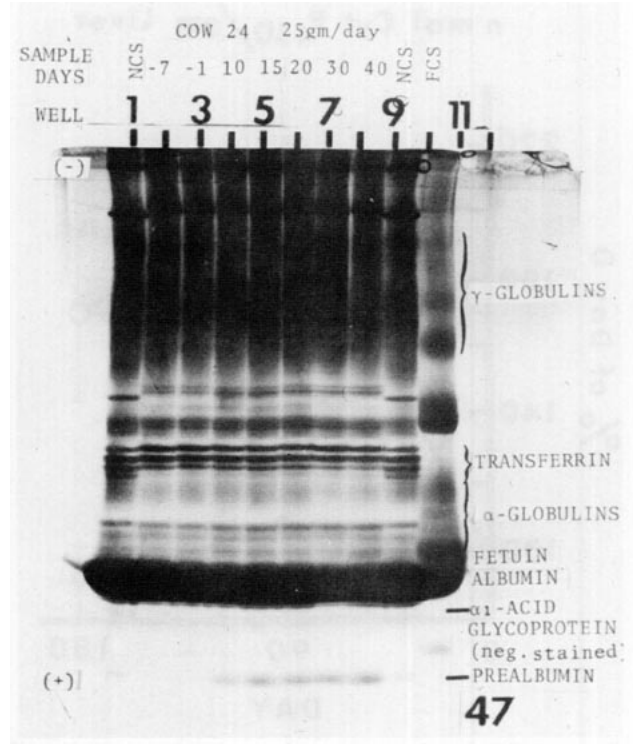

FIGURE 6. Electrophoretic profiles of serum protein in a typical dairy heifer treated with 25 g PBB/day. Days shown are days of PBB dosing, −7 and −1 being samples taken predosing. At day 40, cow 24 was moribund from toxicosis, and necropsy was performed on day 41. Electrophoresis was done with 5-20% linear gradient polyacrylamide slab gels at pH 9.0. Reference samples of normal control adult serum (NCS) and commercial fetal calf serum (FCS) were also run.

mediated uptake of colostral immunoglobulins as a result of PBB exposure in utero. The subsequent protein patterns are normal for the growing calf. This pattern also indicates that maternal exposure to PBB at 250 mg/day does not alter the transfer of immunoglobulins into mammary secretion (colostrum) from serum.

Discussion

These studies show that PBB induced hepatic cytochrome P-450 synthesis in the bovine (Figs. 2 and 3) which was correlated with plasma PBB concentration (Fig. 4). This induction of cytochrome P-450 was probably accompanied by a small but real increase in microsome content of liver tissue (Fig. 1). These increases occurred with a PBB dose of 250 mg/day, which produced no clinical sign of toxicity in the treated heifers, even though the dose was administered for 202 days. Similar induction of hepatic microsomes and cytochrome P-450 was found in rats also given this same PBB mixture. Fire-

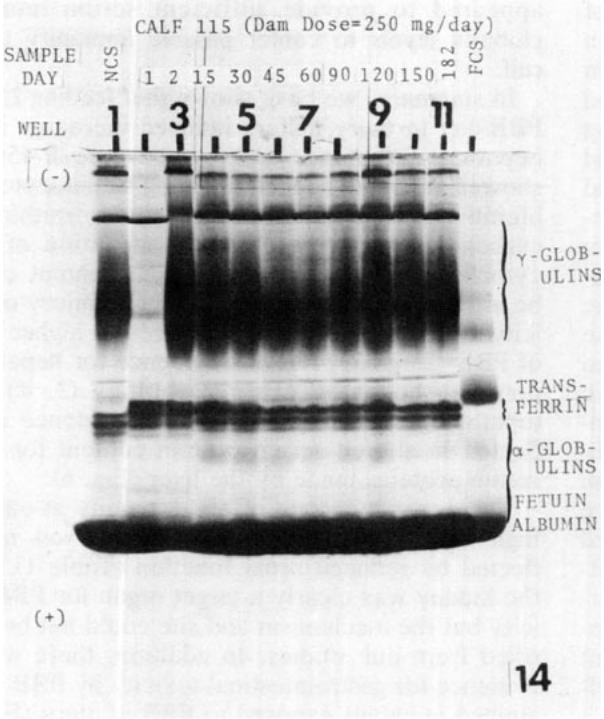

FIGURE 7. *Electrophoretic profiles of serum protein in a typical* calf exposed to PBB in utero. Calf 115 was exposed to PBB while in utero by dosing the dam with 250 mg/day for 60 days in the first trimester of pregnancy. Blood samples were taken at the indicated days of age (day 1 = day of birth), the day 1 sample being taken prior to first nursing of colostrum. The calf was fed PBB contaminated colostrum or milk from its own dam until weaned at 42 days of age. The colostrum or milk was contaminated with PBB which had been previously administered to the dam during pregnancy as described above and entered mammary secretions as a result of increased clearance associated with onset of lactation and utilization of body fat.

Master BP-6 (*7, 8*) and in rats given only hexabromobiphenyl (*6*).

In contrast to the elevation of cytochrome P-450 and microsomes of liver upon exposure to PBB, there was no increase in hepatic ornithine decarboxylase activity. Byus et al. (*21*) reported that PBB as well as 2-methylcholanthrene increased both ornithine decarboxylase and cytochrome P-450 in liver of treated rats. A dramatic increase in ornithine decarboxylase is one of the earliest detectable events leading to cellular proliferation or rapid growth such as occurs in regenerating liver following hepatoinjury (*16, 22, 23*). If elevated ornithine decarboxylase activity is indicative of cellular proliferation in regenerating liver, then it appears that in dairy heifers 250 mg PBB/day did not induce detectable hepatoinjury or liver cell proliferation even though hepatic microsomes and cytochrome P-450 were elevated.

From this, it appears that in the dairy heifer induction of hepatic microsomes and cytochrome P-450 by PBB is dissociated from hepatocyte proliferation and precedes toxicity. Hence, as has been pointed out by others, elevated microsome and cytochrome P-450 content of liver is not necessarily indicative of hepatotoxicity by a xenobiotic compound (*24*). The conclusion drawn from these biochemical analyses that 250 mg PBB/day is not hepatotoxic in dairy heifers is also supported by the clinical diagnosis for these same heifers; no evidence of toxicosis was found in this and a previous study (*4*).

At higher doses of PBB (25 g FireMaster BP-6/day) there was clear evidence of toxicity which was lethal (*2, 12*). At necropsy, histopathological studies of various organs showed, for the liver, enlargement, glycogen depletion of hepatocytes, and sinusoidal dilatation in the liver; and for the kidney, extreme dilatation of collecting ducts and convoluted tubules, with tubular epithelial degenerative changes marked by cloudy swelling, hydropic degeneration, and separation from the basement membrane (*3*). It is unfortunate that no liver tissue was available from these heifers during toxicosis such that microsome and cytochrome P-450 content, and ornithine decarboxylase activity could be examined. For the kidney, in spite of marked histopathological deterioration (*3*), there was no alteration in renal function (*11*) (Table 1). This was a surprising observation and leaves the site and mechanism of renal toxicity by PBB (FireMaster BP-6) undetermined. There is no question that the kidney is a target organ with high levels of PBB exposure.

The serum protein electrophoretic patterns of heifers given 25 g PBB/day for 32–60 days showed significant alteration. Specifically, the serum albumin concentration appeared to decrease while prealbumins and a protein tentatively identified as α_1-acid glycoprotein increased. Both changes occurred during periods of elevated SGOT and SLDH (*4*). There was also a tendency for serum albumin to decline in these heifers during this same period as determined by blood chemical analyses (*4*), thus supporting the conclusions drawn from the electrophoretic profiles of serum protein in this study. The significance of changes in α_1-acid glycoprotein, an acute phase serum glycoprotein made by the liver in response to infection trauma (*20*), and prealbumin is not known at this time, although these changes are probably a further indication of the jury to the liver by high doses of PBB. It is interesting that all of the serum proteins which thus far appear altered by high PBB doses are made by the liver.

Lloyd et al. (*25*) showed a depression in rate of albumin synthesis after partial hepatectomy which was considered to result from a shift in protein synthesis by the hepatocyte away from secreted proteins and toward intracellular protein during post-injury hepatocyte proliferation. Jamieson and Ashton (*20*) describe the increased synthesis and secretion of α_1-acid glycoprotein during liver inflammation. It is generally thought that the in situ tissue damage of inflammation causes increased hepatic synthesis of acute phase proteins (*19*). The role of elevated α_1-acid glycoprotein in acute phase inflammatory responses is unknown, although from these results it appears that xenobiotics in toxic amounts may influence those aspects of the inflammatory responses mediated by the liver. This finding may have ramifications to aspects of disease resistance or immunosurveillance, although we would not expect these ramifications, if indeed there are any, in doses below those producing outright toxicosis with hepatoinjury. This area certainly merits further study for the mechanisms underlying these observations may have important ramifications to both animal and human health effects of xenobiotics.

There was no apparent impairment of the ability of the intestinal mucosa of calves exposed to PBB in utero to transport immunoglobulin into blood as evidenced by the normal electrophoretic pattern of colostrum immunoglobulin absorption into the blood (Fig. 7). The dams of these calves received 250 mg PBB/day or less, doses which have produced no signs of toxicosis in any animal receiving it. It is difficult to assess the in utero dose received by the calf although substantial amounts of PBBs do cross the placenta to enter the fetus (*26*). Polychlorinated biphenyls were shown to enter the fetus and elevate aryl hydrocarbon hydroxylase and cytochrome P-450 or fetal liver (*27*). Thus the fetus is clearly susceptible to xenobiotic toxicosis in utero or at birth. Others have shown that the intestinal mucosa itself has inducible aryl hydrocarbon hydroxylase activity and that this activity is not induced by PCB (*28*), indicating that the drug metabolizing system of intestinal mucosa is susceptible to xenobiotic toxicosis but is different in its control from that of liver. Hence one might expect different tissue sensitivities and responses to xenobiotics. Further, the ingestion of certain xenobiotics may directly affect intestinal mucosa and alter gut function. This apparently did not happen in calves exposed to PBB in utero whose dams received 250 mg PBB/day or less or who subsequently ingested PBB contamined colostrum from the dam. The absorption of immunoglobulin from ingested colostrum into the serum of the calf also appeared to provide sufficient serum immunoglobulin levels to confer passive immunity to the calf.

In summary, we have shown that feeding 250 mg PBB/day to dairy heifers induced increases in the hepatic microsomes and cytochrome P-450 but showed no evidence of inducing hepatocyte proliferative activity as assessed by liver ornithine decarboxylase activities. Hence, elevation of liver cytochrome P-450 and microsome content cannot be interpreted as evidence of hepatic injury or toxicity at this level of PBB. However, at higher doses of PBB (25 g/day) there is evidence for hepatic injury as shown by gross pathology (*2, 4*), histopathology (*8*), and biochemical evidence as reflected in altered serum protein content for those serum proteins made by the liver (Fig. 6).

There was evidence of renal toxicity at only the high dose of PBB (25 g/day) but this was not reflected by reduced renal function (Table 1). Thus the kidney was clearly a target organ for PBB toxicity but the mechanism and site could not be identified from our studies. In addition, there was no evidence for gastrointestinal toxicity by PBB as examined in calves exposed to PBB in utero (Fig. 7), even though the gut is a potential target organ (*28*) and did show histopathological lesions in cows receiving 25 g PBB/day (*3*).

Thus at PBB exposures of 250 mg/day or less, there was no evidence for hepatic, renal, or intestinal toxicity in the dairy heifer. At exposures of 25 g PBB/day toxicity was readily apparent in liver and kidney and deterioration was rapid. These data support the previous conclusion by Durst et al. (*4*) that PBB is a threshold toxin (*29*) in cattle and must be encountered in high concentrations before toxic responses are elicited. Other related work on the effects of PBBs in cattle is summarized in the accompanying papers (*18, 30, 31*). From these studies, it is apparent that large animals are useful and reliable for studying xenobiotic effect, distribution, and clearance. They may also serve to elucidate basic mechanisms of xenobiotic activities which will be useful in understanding their impact on human health.

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