METABOLISM AND NUTRITION

Enhanced Withdrawal of Polychlorinated Biphenyls: A Comparison of Colestipol, Mineral Oil, Propylene Glycol, and Petroleum Jelly with or Without Restricted Feeding[1,2]

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(Received for publication August 8, 1988)

ABSTRACT Meat type chickens were fed a commercial mixture of polychlorinated biphenyl (PCB), Aroclor 1254, at 10 ppm for 14 days, then treated for 21 days to hasten the withdrawal of PCB with either mineral oil (MO), petroleum jelly (PJ), propylene glycol (PG), or colestipol (CO) at 5% of the diet, or at 10% of the diet when restricted to 50% of control intake (50% FR). Whole carcass analyses for PCB revealed that MO + 50% FR reduced PCB to 1.91 mg/bird, or 32% of the body burden (5.96 mg) in nontreated chickens previously fed PCB, whereas those restricted in feed intake by 50% (50% FR) had almost no change (6.44 mg/bird) in body burdens. The PJ, PG, and CO in combination with 50% FR reduced body burdens of PCB to 47, 57, and 77%, respectively, of the control value. When treated with MO, PJ, PG, or CO alone (no 50% FR), chickens had body burdens reduced to only 67 to 90% of control, depending on the compound. Thus, feed restriction was necessary for the MO and PJ to have their greatest effect. Carcass lipid values and body weight gains were markedly reduced by the feed restriction. The CO reduced carcass lipid in nonrestricted chickens by 30%.

(*Key words*: polychlorinated biphenyls, body burdens, feed withdrawal, xenobiotics, carcass lipids)

1989 Poultry Science 68:885–890

INTRODUCTION

The ability to hasten removal of body burdens of xenobiotics from contaminated animals and humans would seem to be highly desirable. First, there is the possibility of aiding humans and animals subject to direct, acute contamination via industrial or agricultural accidents, or exposed to contaminated environments. Second, there are animals and humans previously contaminated with xenobiotics by exposure to products carried along in the food chain. The long-term implications of these low level body burdens are not known; yet, some of these compounds are known to be carcinogenic, teratogenic, or tumor promoters.

Many researchers have attempted to hasten removal of xenobiotics from contaminated animals and humans using altered plane of nutrition, charcoal, phenobarbital, hormones, lipotropic agents, enzyme inducers, or bile-binding resins but with only limited success (Wesley *et al.*, 1966; Wilson *et al.*, 1968; Donaldson *et al.*, 1968, 1971; Smith *et al.*, 1970; Cregar and Kubena, 1971; Egebertson and Davison, 1971; Waibel *et al.*, 1972; Coon and Couch, 1973; Polin and Ringer, 1975; Sell *et al.*, 1977; Cohn *et al.*, 1978; Boylan *et al.*, 1978; Rozman *et al.*, 1982; Polin and Leavitt, 1984). The latter report showed that dosage of the compounds could play a role in their efficacy. Subsequently, almost 70% of the xenobiotics were removed from chickens by combining the partial successes of previous experiments (Polin *et al.*, 1985). The combination of feed restriction and certain chemicals, particularly mineral oil (MO), provided a synergistic effect (Polin *et al.*, 1986), and the procedure appeared to be the most effective of those tried thus far. In subsequent experiments, it reduced, in 21 days, the body burdens of polychlorinated biphenyls (PCB), polybrominated biphenyls (PBB), and hexachlorobenzene (HCB) from chickens by almost 70%; within that time span all of the pentachlorophenol (PCP) was removed (Polin *et al.*, 1986). The compounds, except for PCP, are persistent in adipose tissue, with a characteristically very long term withdrawal. The

[1]Michigan Agricultural Experiment Station Journal Article Number 12701.

[2]Partial financial support received from the Kleberg Foundation, San Antonio, TX.

TABLE 1. *Polychlorinated biphenyls (PCB) in whole carcasses of meat type chickens at Day 14 of feeding diets with 10 ppm Aroclor 1254, and then on Day 21 after withdrawal, during which time chickens were treated with mineral oil (MO), petroleum jelly (PJ), propylene glycol (PG), or colestipol (CO) with or without50% feed restriction*

	No feed restriction			50% Feed restriction		
Compound in diet[1]	n	$\bar{x} \pm$ SE	% of control[2]	n	$\bar{x} \pm$ SE	% of control
		(ppm)			(ppm)	
None	9	2.28 ± .99	100%[bc]	10	3.51 ± 1.16	154%[d]
MO	10	1.69 ± .58	74%[ab]	9	1.08 ± .04	47%[a]
PJ	9	1.63 ± .32	71%[ab]	9	1.73 ± .53	76%[ab]
PG	10	2.06 ± .75	90%[bc]	9	1.98 ± .39	87%[bc]
CO	9	1.72 ± .52	75%[ab]	8	2.53 ± .73	111%[c]
ANOVA summary						
Source of variation	df	Mean square				
Compounds (C)	4	6.29	P<.01			
Feed restriction (FR)	1	2.21	P<.05			
C × FR	4	2.37	P<.01			
Error	82	.46				

[a–d]Means with no common superscript are significantly different (P<.05).

[1]Compounds fed at 5% to *ad libitum*-fed groups and at 10% to feed-restricted groups.

[2]Percentages refer to PCB in carcass in comparison with amounts in nonrestricted controls.

procedure for enhancing withdrawal is based on the nonbiliary excretion of xenobiotics through the intestine (Yoshimura and Yamamoto, 1975); subsequently confirmed by Richter *et al.*, 1977; Boylan *et al.*, 1979; and Richter and Schaefer, 1981. The nonbiliary excretion is presumably based on interference with the absorption of bile salts in the small intestine so that lipid absorption is antagonized. Why the intestine is then capable of secreting or excreting lipophilic substances into the lumen is not known. In addition, the impact this has on fatty acid absorption is also not known. All studies involved with this phenomenon have dealt with xenobiotic removal and not the impact on possible enhanced loss of lipid through the intestine.

Compounds other than MO and colestipol (CO) have shown some activity in hastening withdrawal of xenobiotics; thus, the objective of this study was to compare their efficacy in removing PCB, a very persistent xenobiotic, from chickens.

MATERIALS AND METHODS

Meat type male chickens at 1 day of age were obtained from a commercial hatchery. They were raised to 21 days of age in wire-floored, electrically heated batteries using standard conditions for husbandry and procedures. A commercial starter-grower diet and water were provided *ad libitum*. From 21 days of age, 20 chickens were continued on a commercial grower diet while 80 others were fed the same diet into which PCB (Aroclor 1254, Monsanto, St. Louis, MO) at 10 ppm were incorporated via a minimal amount of corn oil. The same amount of corn oil was also blended into the clean diet. These diets were fed *ad libitum* for 14 days. The diets were removed and the chickens divided randomly into groups of 10. Four groups were fed *ad libitum* diets containing 5% (by weight) of either petroleum jelly (PJ, Fisher Scientific, Detroit, MI), MO (Meijer's Brand, Meijers Stores, Grand Rapids, MI), propylene glycol (PG, Fisher Scientific, Detroit, MI), or CO (Upjohn Co., Kalamazoo, MI). The other four groups were fed diets with the same products at 10% of the diet by weight, but these diets were feed restricted at 50% of control intake (Table 1). This allowed equal daily intakes of the compounds being compared. The restricted feeding functioned to mobilize the adipose tissue, where the xenobiotics are stored in greatest concentrations.

On the 21st day (Day 35 of the experiment), the chickens were weighed individually and then euthanized with excess CO_2. The carcasses (including feathers) were frozen at –21 C until analyzed for PCB as described in a prior publication (Polin *et al.*, 1986) according to the method of the Environmental Protection Agency (Sherma and Beroza, 1979). When the carcasses were to be analyzed, they were

TABLE 2. *Body burdens of polychlorinated biphenyls (PCB) in whole carcasses of meat type chickens at Day 14 of feeding a diet with 10 ppm Aroclor 1254 and then at Day 21 after withdrawal, during which time chickens were treated with mineral oil (MO), petroleum jelly (PJ), propylene glycol (PG), or colestipol (CO) with or without 50% feed restriction*

	No feed restriction			50% Feed restriction		
Compound in diet[1]	n	$\bar{x}$ ± SE	% of control[2]	n	$\bar{x}$ ± SE	% of control
		(ppm)			(ppm)	
None	9	5.96 ± .84	100%[de]	10	6.44 ± .73	108%[e]
MO	10	4.37 ± .48	73%[bcd]	9	1.91 ± .09	32%[a]
PJ	9	4.11 ± .36	69%[bc]	9	2.78 ± .34	47%[ab]
PG	10	5.35 ± .59	90%[cde]	9	3.40 ± .25	57%[ab]
CO	9	3.98 ± .41	67%[bc]	8	4.58 ± 1.14	77%[cd]
ANOVA summary						
Source of variation	df	Mean square				
Compounds (C)	4	10.58	P<.01			
Feed restriction (FR)	1	18.26	P<.01			
C × FR	4	25.65	P<.01			
Error	82	2.375				

[a-d]Means with no common superscript are significantly different (P<.05).

[1]Compounds fed at 5% to *ad libitum*-fed groups and at 10% to feed-restricted groups.

[2]Percentages refer to PCB in carcass in comparison with amounts in nonrestricted controls.

partially thawed, cut into smaller chunks with a Hobart (Troy, OH) meat type bandsaw, and then ground into hamburger consistency (including feathers) in a Hobart 3 hp grinder. Two-gram samples were analyzed for PCB. Duplicate samples of 5 or 10 g were extracted with petroleum ether in soxhlet apparatus for 18 h to remove the lipid that was determined gravimetrically. Body burdens of PCB were calculated from data on body weight and PCB analyses.

The data on PCB, body weight gain, and carcass lipid were analyzed according to Snedecor and Cochran (1968) using ANOVA. Main effects were compounds and effect of feed restriction. Mean differences were determined according to Duncan's multiple range test (1955) and Dunnett's comparison to control (1955). Percentage values were converted to arc sin transformations before statistical analysis.

RESULTS

Chickens euthanized 21 days after withdrawal from a diet with 10 ppm PCB contained average residue concentrations of 2.28 ppm (Table 1). Those restricted in feed intake over the 21 days had average residue concentrations of 3.51 ppm, 54% higher than nonrestricted chickens. The increase was accounted for in retention of PCB (Table 2) and loss of body weight (Table 3). The MO, PJ, and CO, each at 5% of the diet and fed to chickens *ad libitum*, reduced the PCB concentrations to 71 to 77% of that of control (Table 1). The actual reduction was not significant according to a Duncan's multiple range test or Dunnett's comparison to the control. The PG at 5% of the diet also had no significant effect on reducing PCB concentration. However, a comparison of the four treated groups to the control group in the ANOVA resulted in a significant difference because of the larger number of birds in the comparison.

The comparison of the compound plus restricted feeding effect can be made in two ways: a comparison with the residues detected in the restricted birds or with the birds fed *ad libitum*. The more conservative approach is to compare the residues with the *ad libitum*-fed controls. In that comparison, chickens restricted in feed intake at 50% (50% FR) while being fed the compounds at 10% of the diet had significantly (P<.01) lower body residue concentrations when given MO. The 1.08 ppm value was 47% of the control value (Table 1), or a reduction of 53%, as compared with a reduction of 69% when compared with the value for restricted controls. The PJ was the next most effective compound, reducing the PCB in FR chickens to 76% of those in the *ad libitum*-fed control, or 49% of those of the restricted control, a value not much different from those of birds fed without restriction of the diet. The CO and PG were not effective in

TABLE 3. *Gain in weight and percentage carcass fat by Day 21 of withdrawal of polychlorinated biphenyls (PCB) and average feed intake of meat type chickens during the withdrawal period. Treatments were mineral oil (MO), petroleum jelly (PJ), propylene glycol (PG), or colestipol (CO).*

Variable	Compounds in diet[1]	*Ad libitum feeding*		50% Restricted feeding	
		(n)		(n)	
Body weight gain, g	None (formerly fed PCB)	10	1,221 ± 162[a]	10	368 ± 100[b]
	MO	10	1,220 ± 109[a]	9	372 ± 170[b]
	PJ	9	1,118 ± 256[a]	10	203 ± 131[b]
	PG	10	1,257 ± 92[a]	10	428 ± 137[b]
	CO	9	1,077 ± 142[a]	9	363 ± 157[b]
	None (clean feed always)	9	1,100 ± 154[a]	9	415 ± 160[b]
Lipid in carcass, % (arc sin transformation)	None (formerly fed PCB)	10	10.7 ± 3.3[a]	10	6.3 ± 1.2[bc]
	MO	10	11.4 ± 2.1[a]	9	4.6 ± 1.6[cd]
	PJ	9	11.2 ± 2.8[a]	10	4.2 ± .8[d]
	PG	10	11.3 ± 2.1[a]	10	7.0 ± 1.7[b]
	CO	9	7.3 ± 2.5[b]	9	4.1 ± 1.6[d]
	None (clean feed always)	9	9.7 ± 1.7[a]	9	5.9 ± 1.9[bc]
Feed intake, g/bird/day[2]	None (formerly fed PCB)	10	161 (100)	10	89 (55)
	MO	10	166 (103)	9	94 (58)
	PJ	9	162 (101)	10	74 (46)
	PG	10	158 (98)	10	80 (50)
	CO	9	169 (105)	9	95 (59)
	None (clean feed always)	9	164 (102)	9	88 (55)

[a–d]Means with no common superscripts within a criterion are significantly different (P<.05).

[1]Compounds fed at 5% to *ad libitum*-fed groups and at 10% to feed-restricted groups.

[2]Numbers in parentheses represent intake as a percentage of that of controls.

reducing the PCB concentrations to values significantly less than those in the *ad libitum* control, although the values were significantly less than those of the restricted controls. There was a significant interaction (Table 1) accounted for by the marked effect of feed restriction in combination with MO to enhance PCB withdrawal.

The data on body burdens of PCB include the body weight in the calculation; they reflect what is actually remaining in the chickens. In this case, PCB were detected at 5.96 mg/bird in the *ad libitum*-fed chickens 21 days after PCB withdrawal, a value not much different than the 6.44 mg detected in the birds fed 50% FR. The CO, PJ, and MO without 50% FR reduced body burdens 67, 69, and 73%, respectively, of the *ad libitum*-fed control body burdens. Ninety percent of the PCB was still in the chickens treated with PG. Again, the ANOVA revealed a significant treatment effect and interaction that on closer examination involved primarily the groups given the combination treatment of compound and 50% FR. The chickens treated with MO and 50% FR had the lowest body burdens–about 30 to 32% of the controls that were fed *ad libitum* or 50% FR–and that marked change accounted for the significant (P<.01) interaction (Table 2). The PJ and PG in combination with 50% FR were as effective in reducing the body burdens as MO, but the values of 2.78 and 3.40 were only 47 to 57% of the control value. The CO was the least effective of the compounds in reducing body burdens when used with 50% FR.

Chickens fed *ad libitum* gained 1,100 g in 21 days if they had not been fed PCB for 14 days prior to the withdrawal period and 1,221 g (nonsignificant difference from control at P>.05) if they had been fed PCB. Chickens fed *ad libitum* and treated with the various compounds grew as well as these controls (Table 3). Chickens restricted in feed intake to 50% of the controls gained 415 g if not previously fed PCB and 368 g if previously fed PCB (values are not significantly different, P>.05). Weight gains among the groups treated with compounds plus 50% FR were not significantly different.

Lipid contents of the *ad libitum*-fed chickens were 9.7 and 10.7% of body weight, with values for the birds fed MO, PJ, and PG ranging from 11.2 to 11.4% (Table 3). These values were not significantly different from those of the controls. The lipid content of carcass, at 7.3%, was significantly lower (P<.05) in chickens fed 5% CO than in the other *ad libitum*-fed chickens. Feed restriction markedly reduced carcass lipid content, to about 60% of the nontreated values. Chickens fed CO + 50% FR shared the lowest lipid content with the treatments of PJ + 50% FR and MO + 50% FR; carcass lipid values ranged from 4.1 to 4.6% of body weight.

Feed intake for the *ad libitum*-fed chickens ranged from 158 to 169 g/bird per day during the 21 days of withdrawal. The intake of feed-restricted birds ranged from 74 to 95 g/bird per day. The lowest values were for restricted birds fed diets containing PJ and PG. The variation in these values reflects the day-to-day attempts to feed at 50% of the control. The body weight gains of the group fed PG + 50% FR reflect to some extent the lower than average feed intake for the restricted groups (Table 3).

DISCUSSION

This study confirmed the data obtained with PBB (Polin *et al.*, 1985): body burdens of PCB in birds fed Aroclor 1254, were markedly reduced within 21 days by using MO + 50% FR. The PJ + 50% treatment was almost as effective as MO + 50% FR. When only the residue concentrations in the carcass were considered, MO + 50% FR was again the most effective of the treatments, presumably reflecting the nonbiliary intestinal secretion as described by Yoshimura and Yamamoto (1975) and enhanced by the FR effect to force withdrawal of the xenobiotic from lipid stores.

Body burdens of PCB of control chickens that were restricted in feed intake were practically unchanged from those of the untreated controls. Thus, feed restriction by itself was not an effective procedure to remove the PCB. When used in combination with MO, a synergistic effect was detected. Thus, feed restriction was decidedly necessary to mobilize the adipose tissue, as indicated by the markedly reduced lipid values in the feed-restricted chickens, to get the PCB into the circulatory system and then to the intestinal site for removal. Some differences are evident in the way the various compounds were effective in hastening the withdrawal of the PCB. For example, CO was as effective as MO and PJ when not used with feed restriction. But, CO reduced lipid in the carcass whereas MO and PJ had no such effect. One would have thought that if carcass lipid (as a storage site for the PCB) were significantly reduced while the compound was having some effect in hastening the withdrawal of the xenobiotic, then the compound would exhibit greater withdrawal effectiveness.

Lower carcass lipid, *per se*, does not appear to be the answer to the enhanced synergistic effect obtained above that induced by the compounds themselves. First, one has to consider how that carcass lipid was reduced. Colestipol acts in the intestine to reduce lipid absorption by its antibiliary action. Thus, it presumably would reduce reabsorption within the intestine of the small amounts of PCB excreted through the bile. However, feed restriction reduced lipid reserves in the body where most of the PCB were stored, forcing the PCB into the circulatory system, from which the antibiliary pathway for xenobiotic secretion across the intestinal wall can occur. Second, restriction alone, based on data from the restricted controls, did not remove PCB in this experiment. So, carcass lipid removal by feed restriction alone was not effective in removing PCB unless a compound or dietary component was present to act in the nonbiliary intestinal pathway. The mechanism for this excretion or secretion across the intestinal wall and into the lumen is not known. What is known is that bile acid removal from the intestinal lumen, or sequestering by some compound, physiological deficiency, or rerouting of bile activates the process.

There are reports in which feed restriction was partially effective in reducing xenobiotic residues. For example, rats fasted at 25% of control intake had somewhat reduced PCB (Matthews and Anderson, 1975; Wyss *et al.*, 1982). Also, others have reported some reduction by feed restriction of DDT (Donaldson *et al.*, 1968), dieldrin (Sell *et al.*, 1977), PBB (Polin *et al.*, 1985), and HCB and PCP (Polin *et al.*, 1986). In substantiation of the data in this report about chickens, rats fasted at 75% of controls showed no reduction of HCB (Villenueve, 1975). There are times when experimental procedure or the type of animal used will make a difference in the effectiveness of the FR treatment. Why this is so is not known, unless the ingredients used in the diet may be involved.

On a practical basis, the techniques used in this experiment are valid procedures for decontaminating broiler chickens during their grow-out period if they are contaminated to a major extent with a persistent xenobiotic. Weight gain would be markedly reduced, and the bird would not be entirely free of xenobiotic. However, contaminations near allowance limits may lend themselves to removal sufficient to allow the birds on the market. Most important is that the research demonstrates that a system is operating in the chicken allowing a hastening of the withdrawal of xenobiotics. Next it is necessary to find the way to enhance that system to remove all of the xenobiotic, or to remove enough to get the moderate to high contaminating levels below allowance limits. In addition, an amount of feed restriction less than 50% FR must be found to produce a comparable reduction of the xenobiotics yet allow greater body weight gain. The use of compounds to turn over adipose tissue at a faster rate may serve as an approach to hasten withdrawal of xenobiotics in the presence of compounds effective in the nonbiliary intestinal pathway for secretion of xenobiotics. In that approach, feed restriction would not be used and the chicken would gain in weight according to its potential.

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