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Responses of Nonhuman Primates to a Polybrominated Biphenyl Mixture

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In a series of experiments, rhesus monkeys have been given in their diets 0.3, 1.5, and 25 ppm of a commercial polybrominated biphenyl (PBB) (as FireMaster FF-1). The seven adult female monkeys receiving 0.3 ppm PBB have been on the treatment regime for 15 months and have consumed over 22 mg of PBB. During the initial 6 months of exposure, they lost weight and 2 of the animals develop sterile abscesses. At 6 months, 4 of the 7 animals had flattened and lengthened serum progesterone peaks. This change was correlated with an increase in length of their menstrual cycles. After 6 months of PBB exposure, the animals were bred. Two of the 7 animals showed excessive and prolonged implantation bleeding. Two abortions and 5 live births were recorded. All of the experimental infants were smaller than the controls at birth. The animals receiving a diet containing 1.5 ppm PBB for 36 weeks (total intake 70 mg) have shown a moderate weight loss and decrease in serum cholesterol. Similar changes have also been recorded in the group given the 25 ppm PBB diet for 14 weeks (approximately 500 mg total intake). In addition, these animals have also developed a hyperplastic gastritis.

Polybrominated biphenyls (PBBs) as an environmental contaminant were brought to public attention as a result of the accidental mixing of a commercial flame retardant, FireMaster (Michigan Chemical Corp., St. Louis, Michigan), into livestock feed in Michigan in July 1973 (*1*). Affected dairy herds initially showed anorexia, with a 40% drop in milk production. Weight loss, lameness, and increased frequency of urination and excessive lacrimation were noted. Histologically, liver changes in the cattle included fatty metamorphosis and amyloidosis. There were also reports which indicated kidney changes including pigment nephrosis and acute, subacute and chronic interstitial nephritis. During pregnancy there was loss of appetite and leg weakness. At parturition weak or dead fetuses were common. Cows bred 4 to 6 weeks prior to PBB exposure returned to estrus (*2*).

In farm families exposed to high levels of PBBs, there have been reports of unusual rashes, gastrointestinal disturbances, increased fatigability, lethargy, altered immune function and white blood cell disorders and neurological complaints (*3, 4*). Reproductive abnormalities in exposed women have not yet been fully evaluated. Studies show, however, that PBBs, like polychlorinated biphenyls (PCBs), affect steroidogenesis. Milk production, egg production, fertility and spermatogenesis have been found to be adversely affected in various species (*2, 5, 6*). In addition, PBBs have also been shown to pass across the placental barrier and be excreted in the milk of lactating mothers (*7, 8*).

In order to determine the effects of PBBs on primates under controlled conditions, rhesus monkeys were fed diets containing PBBs at levels to which the human population may have been exposed. The data obtained in these experiments are the subject of this report.

Materials and Methods

Group Receiving 0.3 ppm PBB

Fourteen adult female rhesus monkeys with an average weight of 6.0 kg were used in this experiment. The monkeys were housed in a controlled environment simulating the light, humidity, and temperature conditions of the breeding season for

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these feral animals in India. All monkeys had regular menstrual cycles for at least two years prior to the initiation of this experiment, and all had previously given birth to viable infants. Hemograms and serum chemistry values which included SGPT, cholesterol and total lipids were also determined to be normal.

Seven of these animals were placed on a diet containing 0.3 ppm of the FireMaster FF-1 diet for 66 weeks. The diet was prepared by dissolving the PBB in corn oil and then mixing with commercially prepared monkey chow. The seven animals in the control group received a diet prepared by dissolving an equal amount of corn oil in the monkey chow. After mixing the diets were pelletted. The animals received 200 g/day of either the experimental or control diets which were supplemented with fresh fruit twice weekly and water *ad libitum*.

Throughout the course of the experiment, the animals were observed daily for clinical changes. Complete hemograms and serum chemistry determinations as enumerated previously were performed at 1, 3, 6, 9, 12, and 15 months. Body weights were recorded biweekly.

In order to determine levels of serum progesterone and 17-β-estradiol, 5 cm^3 blood samples were drawn from the animals by femoral venipuncture daily through a complete menstrual cycle initially and after 6 months on the experimental diet. In addition, blood samples were obtained daily for 15 days of the cycle at 1 and 3 months. This blood was centrifuged at 2000 rpm, and serum removed and stored at −20°C until radioimmunoassay was performed on the entire cycle. Radioimmunoassays were conducted on the serum for progesterone and 17-β-estradiol according to the procedures of Barsotti (*9*) and Hotchkiss et al. (*10*).

After 7 months on the experimental or control diets, the 14 females were bred. Ovulation was predicted from observation of menses and previous menstrual cycle length (*11*). The females were housed with control males for 5 days during the appropriate time of ovulation. Pregnancy tests on females were performed according to the procedures outlined by Wilson et al. (*12*). The findings were confirmed by rectal palpation of the uterus at 40 days of gestation. All infants were weighed, measured, and carefully examined for any abnormalities at birth, one week after birth, and biweekly thereafter. These infants were permitted to remain with their mothers, and their only source of nourishment was through nursing.

Group Receiving 1.5 ppm PBB

Three adult rhesus females weighing an average of 6.0 kg were fed 200 g daily of a monkey chow preparation containing 1.5 ppm FireMaster FF-1 for 9 months. All other procedures employed for these animals were similar to those described for the 0.3 ppm group. Blood samples for hemograms and serum chemistry were drawn bimonthly and a liver (left lateral lobe) and stomach biopsy (3 cm from pyloric sphincter) was performed at 9 months.

Group Receiving 25 ppm PBB

A 3.0 kg juvenile female and a 9.0 kg adult male rhesus monkey were fed 200 and 300 g daily, respectively, of a diet containing 25 ppm PBB for 14 weeks. Hemograms were conducted monthly on both animals. After 10 weeks, the experimental male monkey and a control monkey were anesthesized with a general anesthetic (Vetalar, Parke-Davis) and intubated intragastrically with a suspension of barium sulfate. These animals were subsequently radiographed at 30 minute intervals following the procedure. After 12 weeks on the experimental diet, a biopsy was performed on the liver and stomach wall in a manner similar to that previously described for the animals given 1.5 ppm PBB.

Results

Group Receiving 0.3 ppm PBB

During the first 7 months prior to breeding, female rhesus monkeys ingested an average of 10.5 mg PBB. Despite normal food consumption, the experimental group lost an average of 7.4% of their initial body weight. Total intake for the 66 weeks was an average of 23.0 mg PBB. During the entire 66 week period, clinical blood data remained within normal limits, and their general appearance was unaltered with the exception of two animals that developed sterile abscesses on the thighs which healed spontaneously.

After 6 months on the diet, menstrual cycles were somewhat lengthened as compared to their control cycles in four of the animals (28 days vs. 31 days), and these animals showed a corresponding flattening of the progesterone peak. All control animals conceived after one to three breedings and delivered normal appearing infants. All experimental animals conceived after one to four breedings. Two of these animals showed long (12–16 day) periods of implantation bleeding but did maintain pregnancy. One animal aborted a mummified fetus at 146 days of gestation, and one gave birth to a stillborn infant at 154 days. The remaining five animals delivered normal-appearing, but small (455 ± 34 vs. 519 ± 53) infants between 156 to 165 days gestation (Table 1). In addition, the rate of weight gain in these infants was less than that of the control infants (Table 1).

Table 1. Birth and growth weights for infant rhesus monkeys whose mothers had received 0.3 ppm FireMaster FF-1 in the diet for 66 weeks.

Age, mo.	Weight, g[a]	
	Control	Experimental
Birth	519 ± 53 (7)	455 ± 34[b] (5)
1 month	666 ± 89 (7)	518 ± 67[b] (5)
2 months	750 ± 65 (7)	591 ± 82[c] (4)
3 months	901 ± 48 (7)	706 ± 62[c] (2)

[a] Number in parenthesis represents number of animals per group. Values are means ± standard deviations.
[b] Significant difference from controls as determined by Student's t-test, $p < 0.05$.
[c] Significant difference from control, $p < 0.01$.

Group Receiving 1.5 ppm PBB

The mean PBB intake for the 38 weeks that the three experimental animals were on the diet was 77 mg. Serum cholesterol decreased in all of the animals in the 1.5 ppm PBB group over the first 6 months (221 ± 28 vs. 127 ± 25). Two of the animals showed moderate weight loss, and the animals also showed a slight increase in serum SGPT, while other clinical parameters remained unchanged.

The stomach biopsy performed at nine months showed no gross or histological changes. In the liver biopsies the hepatocytes were enlarged with moderate fatty infiltration.

Group Receiving 25 ppm PBB

Total intake of PBB for the 14 week period was 590 mg for the male and 455 mg PBB for the female. The adult male on the 25 ppm diet showed steady weight loss, while the juvenile female maintained a constant weight.

Following the intragastric intubation with barium which occurred during the tenth week of exposure, a series of radiographs were taken of the male experimental animal and a control animal. In the control animals, a large percentage of the barium had passed through the stomach within 1 hr following intubation. By the 3 hr, practically no barium was present in the stomach of the control animal. However, in the experimental animal, all of the barium was in the stomach one hour after intubation, and it required 24 hr for all of the barium to be eliminated from the stomach (Figs. 1 and 2).

Stomach and liver biopsies taken from the two experimental animals after 12 weeks on the experimental diet revealed a decided enlargement of the hepatic cells with an obvious encroachment on the sinusoidal spaces. Ultrastructural evaluations of the hepatocytes revealed a decided increase in smooth endoplasmic reticulum and an increase in frequency of lipid droplets.

The stomach mucosa of the male animal showed a proliferation of the mucous-secreting cells (Figs. 3 and 4), focal areas of infiltration of chronic inflammatory cells, and isolated penetrations of the gastric mucosa through the muscularis mucosae into the underlying submucosa (Fig. 5). The changes in the gastric mucosa were also recorded in the juvenile female monkey.

Discussion

Under the outlined experimental conditions, there are indications that levels of PBB (FireMaster FF-1) ranging from 0.3 to 25 ppm cause a decrease in body weight despite an unmodified level of food intake. Similar observations have also been observed in cattle (*2*). During the course of this study there have been no permanent changes in the hemograms and serum chemistry of the experimental animals similar to those observed in cattle that were heavily exposed to PBBs, with the exception of decreased serum cholesterol observed in the animals of the 1.5 ppm group (*1*). This, however, is not surprising in that obvious changes in peripheral blood of animals exposed to the halogenated hydrocarbons are manifested only after obvious signs of intoxication have arisen (*13*).

Perhaps one of the most significant findings to date in this study are the suggestions of an endocrine imbalance in the animals exposed to 0.3 ppm PBB for 6 months. The lengthening of the menstrual cycle, decreased levels of progesterone and estradiol, along with a flattening and prolongation of the levels in the serum similar to that recorded in the PBB-exposed animals have been shown to be associated with reduced conceptions and abortions in primates exposed to low levels of polychlorinated biphenyls (*7*). Thus, the possibility exists that exposure to these low levels of PBB for a longer period or to higher levels for shorter periods may conceivably be associated with more dramatic changes in the reproductive capability of the PBB-exposed animals.

Secondly, there are definite suggestions from radiological and histological data that the PBBs, like the PCBs (*13*), produce a proliferative gastritis. The slow passage of the barium through the stomach is likely related to reduced diameter of the pylorus at the sphincter due to mucosal hyperplasia and a reduced gastric motility. Although in the present study the gastric lesions had not progressed as far as those recorded in PCB-exposed primates, at the early stages of development the changes are similar.

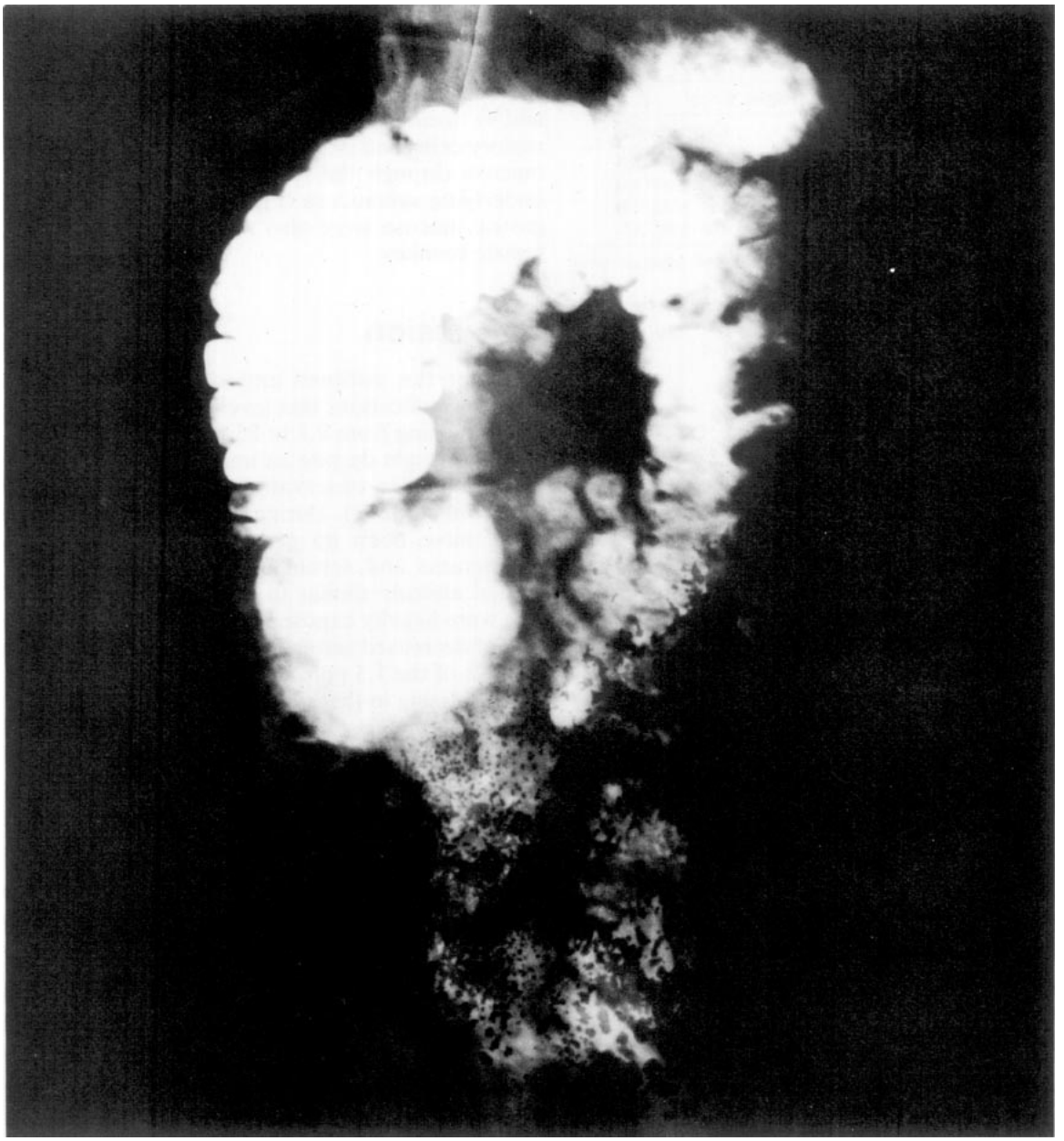

FIGURE 1. Radiograph of a control monkey intubated with barium sulfate 5 hr previously. Note the presence of barium in the intestinal tract.

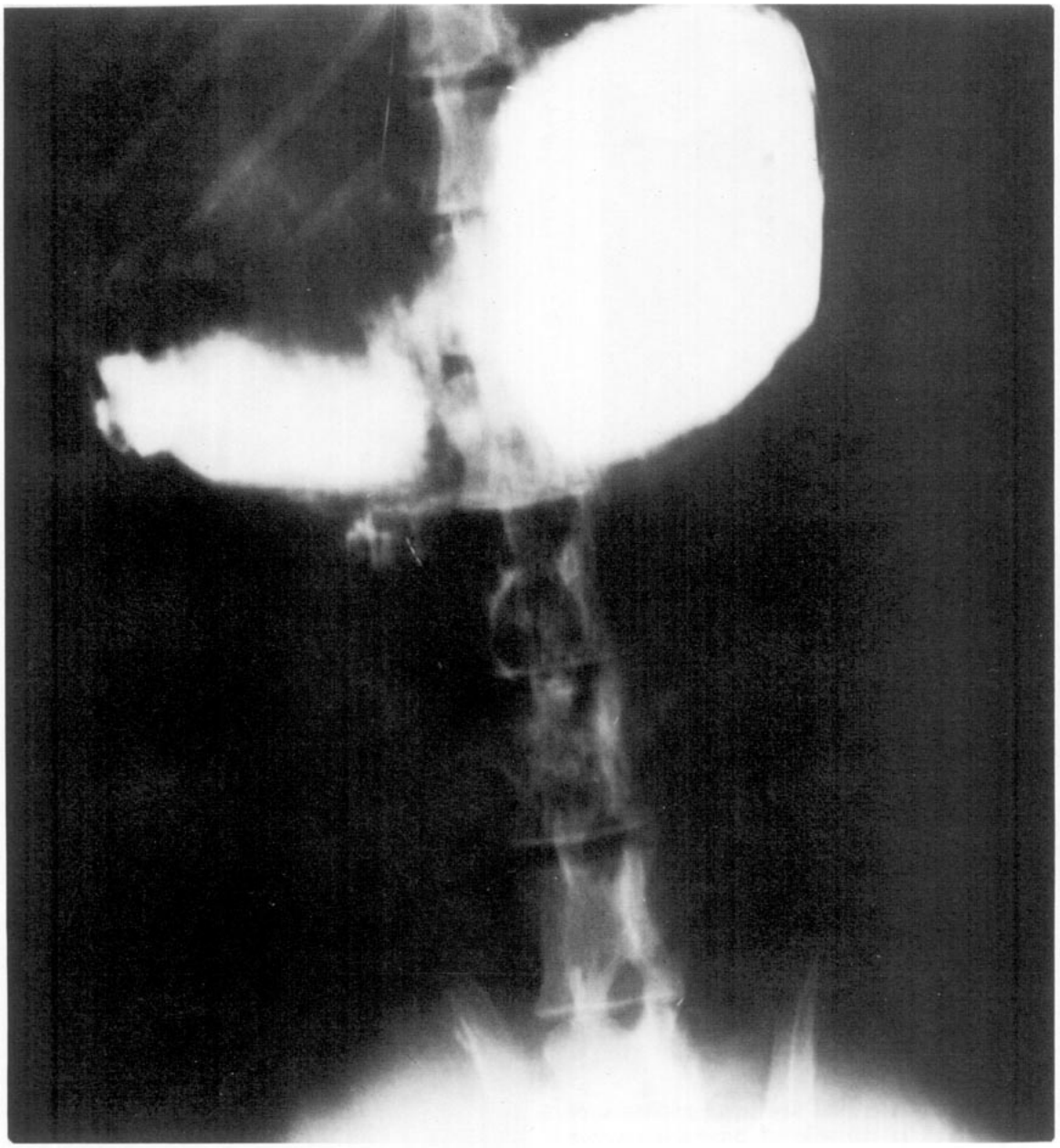

FIGURE 2. Radiograph of a monkey that had received a diet containing 25 ppm PBB for 12 weeks; barium sulfate had been intubated 5 hr previously. Note the persistence of barium in the stomach.

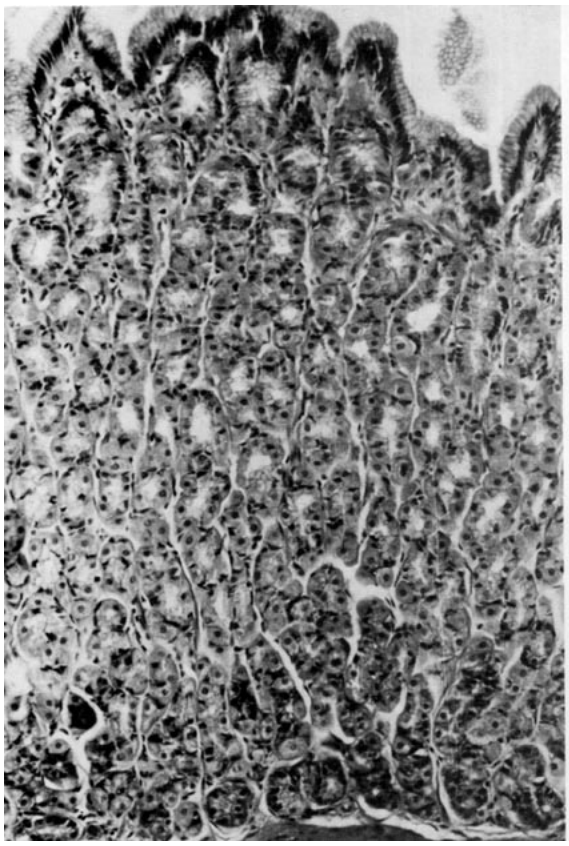

FIGURE 3. Gastric mucosa of a control monkey. Section obtained 3 cm from pyloric sphincter along the greater curvature of the stomach. Note the abundance of mucous-secreting cells along the luminal surface. Hematoxylin and eosin stain; 50×.

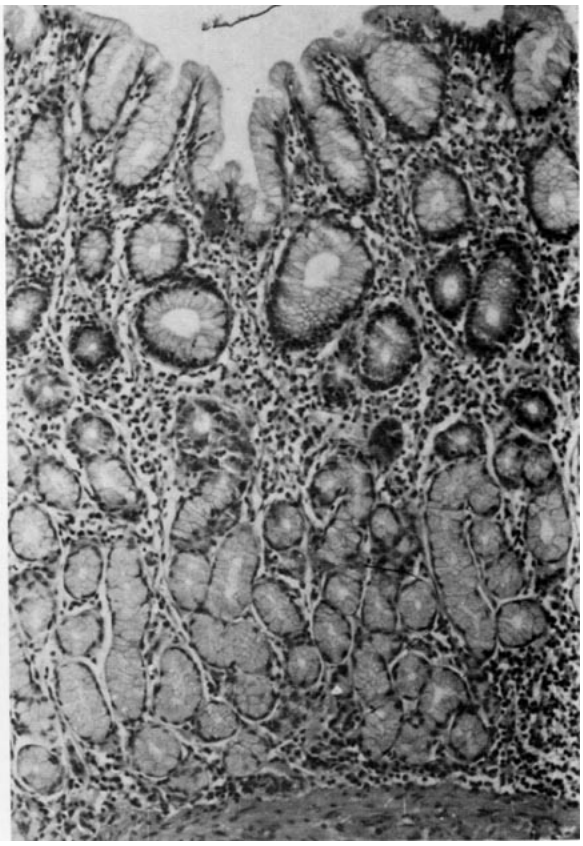

FIGURE 4. Gastric mucosa of a monkey that had consumed a diet containing 25 ppm PCB for 12 weeks. Section taken 3 cm from pyloric sphincter along the greater curvature. Note the predominance of mucous secreting cells throughout the mucosa. Hematoxylin and eosin stain; 50×.

There are indications that low level exposure of the adult females is capable of producing some fetotoxicity. Infants born to mothers that were exposed to PBBs during gestation were consistently smaller than control infants even though the food intake of the two groups was similar. The low birth weight and weight gains observed in the experimental infants are especially interesting when compared to data recorded in infant monkeys born to mothers receiving 2.5 or 5.0 ppm PCB in ther pre- and postpartum diets (*13*). Even though the PCB-exposed mothers had consumed levels of PCBs 8 to 16 times higher than the PBB mothers in the present experiment, infants showed similar impeded growth rates.

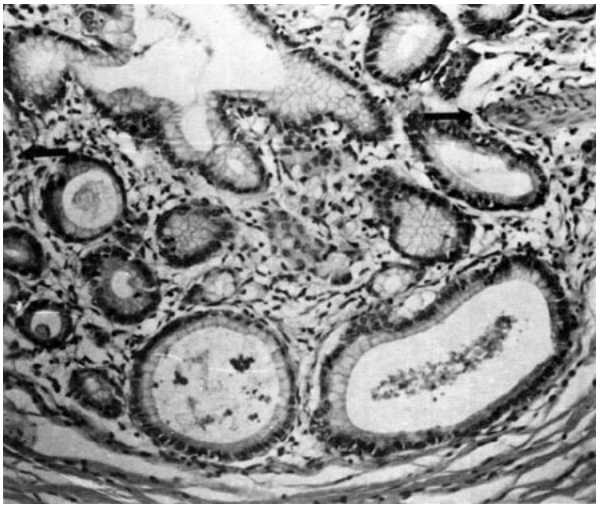

FIGURE 5. Gastric epithelial cells have penetrated the muscularis mucosa (arrow) and displaced the underlying submucosal tissue. Tissues obtained from monkey given 25 ppm PBB for 12 weeks. Hematoxylin and eosin stain; 46×.

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