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Endocrine Effects of Chlorinated Hydrocarbons in Rhesus Monkeys[1]

W. F. MÜLLER*, W. HOBSON†, G. B. FULLER, W. KNAUF†, F. COULSTON†, AND F. KORTE*

* *Institut für Oekologische Chemie der Gesellschaft für Strahlen- und Umweltforschung mbH, Munich, Federal Republic of Germany, and the International Center of Environmental Safety, P.O. Box 1027, Holloman Air Force Base, New Mexico 88330, and the † Institute of Comparative and Human Toxicology, Albany Medical College, Albany, New York 12208*

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After the rhesus monkey was demonstrated to be a suitable model for man in both metabolic and endocrinological studies, effects of hexachlorobenzene (HCB) and polychlorinated biphenyls (PCB) on the pattern of sexual hormones in cycling female rhesus monkeys were investigated. After confirmed ovulation, four adult female rhesus monkeys were treated during the following cycle with 4 mg/kg/day of HCB, and four other monkeys were treated with the same dose of Clophen A 30. Ovulation was blocked in three PCB-treated and one HCB-treated monkeys. Whereas the levels of luteinizing hormone and follicle-stimulating hormone did not seem to be changed directly by the treatment, low estrogen levels were found during the anovulatory cycles. Studies with PCB- and HCB-treated superovulated rats indicated interaction of the chemicals with ovarian steroidogenesis. Altered hepatic steroid metabolism may also cause low estrogen levels in treated animals.

Under the joint program "Ecologic-Toxicological Effects of Environmental Chemicals in Non-Human Primates and Other Laboratory Animals," the Institut für Oekologische Chemie der Gesellschaft für Strahlen- und Umweltforschung mbH, Munich, together with scientists of the Albany Medical College, is conducting studies of the metabolism and toxicokinetics and the immunological and endocrine effects of chlorinated hydrocarbons such as dieldrin, polychlorinated biphenyls (PCB), and hexachlorobenzene (HCB). The objective of the cooperative program is to achieve a better understanding of the chemicals' effects on the primate organism by simultaneous studies of different chemical and toxicological aspects and by joint critical evaluation of the findings.

After Hobson demonstrated in previous studies (Hobson *et al.*, 1977) the value of the rhesus monkey for endocrinological investigations, a comparative study of the metabolism of dieldrin in mice, rats, rabbits, rhesus monkeys, and chimpanzees was designed to determine the value of the primate in comparison to that of lower mammals for the extrapolation of results to man. (Müller *et al.*, 1975).

The two most important metabolites of dieldrin in mammals are formed by opening of the epoxide to 4,5-aldrin-*trans*-diol and by direct oxidation of the unchlorinated methylene bridge to 12-hydroxy-dieldrin (Fig. 1). The latter product has been reported

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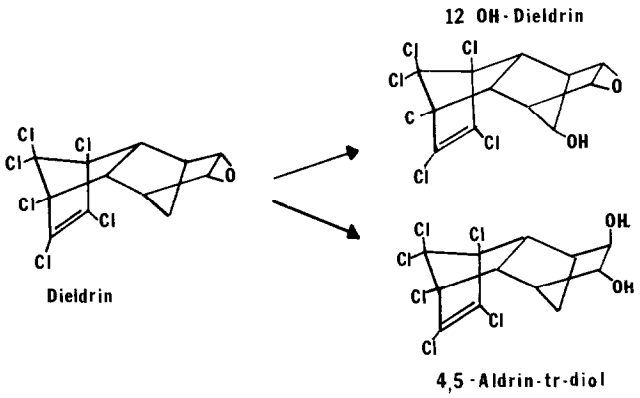

FIG. 1. Dieldrin and its major metabolites.

as the major human metabolite (Richardson, 1971). In our study, the animals were given 0.5 mg of [^{14}C] dieldrin/kg of bodyweight as a single oral dose; then we monitored the radioactivity of the total excreta and the metabolite ratio for 10 days.

The results, summarized in Table 1, showed that both the primate species and the rat were comparable to man, and that the mouse and the rabbit, predominantly excreting the diol, are not very useful as models for man in this respect. A subsequent chronic feeding study of rhesus showed that this species is similar to man in its storage and excretion pattern, whereas the rat, with its higher rate of turnover, is markedly different from the primates.

Parallel to the endocrinological studies with PCB and hexachlorobenzene, the metabolism, storage, and excretion of ^{14}C-labeled chlorinated biphenyls and HCB were studied in rhesus (Felt, 1977; Rozman, 1975). Di- and trichlorobiphenyls were rapidly hydroxylated, conjugated, and excreted; chronic feeding of 5 ppm in the daily diet did not result in any essential storage. A pentachlorobiphenyl(2,4,6,2′,4′) was metabolized and excreted much more slowly; chronic feeding of 5 ppm in the daily diet resulted in adipose tissue concentrations of up to 80 ppm in the male and up to 20 ppm in the female monkey.

TABLE 1

RADIOACTIVE MATERIAL EXCRETED WITHIN 10 DAYS AFTER SINGLE ORAL ADMINISTRATION OF 0.5 mg OF [^{14}C]DIELDRIN/kg OF BODY WEIGHT (PERCENTAGE OF ADMINISTRATION DOSE)

	Mice		Rats		Rabbits		Rhesus Chimpanzee	
	Male	Female	Male	Female	Male	Female	Male	Female
Dieldrin	5.5	1.2	0.8	2.8	0.3	0.5	9.0	3.2
12-OH-Dieldrin	13.0	7.5	8.8	4.6	—	0.2	9.4	2.0
Aldrin-*trans*-diol	20.0	26.0	2.3	2.4	1.5	2.0	2.0	1.1
Total	38.5	34.7	11.9	9.8	1.8	2.7	20.4	6.3

Hexachlorobenzene is metabolized only very slowly to pentachlorobenzene and pentachlorophenol. The lymphatic system plays a major part in the uptake of HCB from the gastrointestinal tract and in its distribution in the body. The storage rate is very high: feeding of 1 ppm of HCB in the diet results, after 2 years, in adipose tissue concentrations of up to 20 to 30 ppm.

During the chronic feeding studies with HCB and PCB, serum levels of the sexual hormones FSH, LH, prolactin, estrogen, progesterone, and testosterone as well as of the enzymes SGOT, SGPT, and LDH were monitored. At the given dose levels, the values of all these parameters showed no significant difference from normal values for rhesus. Likewise, hemoglobin, hematocrit, red and white blood cells, bilirubin, BUN, cholesterol, total protein, creatin, and electrolytes in the blood remained within normal limits.

In a dose-ranging study of the investigation of endocrinologic effects of HCB, female rhesus monkeys were given daily doses of 8, 16, 32, 64, or 128 mg of HCB/kg of body weight for 60 days (Iatropoulos, 1977). Subsequent autopsy of the animals revealed, in addition to damage of dose-dependent severity in the central nervous system, thymus, kidney, and liver, remarkable changes of the ovaries as compared to 15 control ovaries from untreated animals (Table 2). At the lowest dose level there was a change from the cuboidal, unilayer germinal epithelium of the control ovaries to a tall, columnar unilayer form. With increasing dose levels (32 and 64 mg/kg), the germinal epithelium appeared pseudostratified; however, in the highest dose group the columnar form was observed. In all HCB-exposed monkeys, the ratio of corpora lutea to primary follicles was greater than that of the controls; this parameter appeared to be dose dependent. The ovarian cortices of the animals receiving 128 mg/kg consisted predominantly of dense stroma and contained 80% fewer primary follicles. The cortices of the lower dosed animals showed varying degrees of degeneration and marked reduction in the number of primary follicles.

In a dose-ranging study with Clophen A 30, a mixture of mainly di-, tri-, and tetrachlorobiphenyls, which was given at doses of 4, 16, and 64 mg/kg/day for 28 days, no ovarian changes were found. However, Allen (1974) reported that PCBs cause greatly reduced fertility in female rhesus monkeys. Therefore, PCBs were included in further endocrinology studies.

On the basis of the histopathological findings, the following hypothesis was formed: The HCB is somehow acting on the ovary by an endocrine mechanism. Four possibilities were taken into consideration:

(1) Changes in hypothalamic function. There could be increased or decreased secretion of gonadotropin releasing hormone (GnRH), possibly mediated by increased or decreased sensitivity to steroid feedback. Changes in levels of GnRH would act on the pituitary, increasing or decreasing the secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which, in turn, would affect the ovary directly.

(2) Changes at the pituitary level may include a change in sensitivity to GnRH, change in sensitivity to steroid feedback, or direct alteration of secretion of LH or FSH.

(3) Direct effects on the ovary are possible by alteration of steroid synthesis, direct morphological damage to primary follicles, alteration of gonadotropin receptors, and alteration of steroid receptors.

(4) The fourth possible level of HCB interaction could be altered liver metabolism:

TABLE 2

EFFECTS OF HCB ON OVARIES OF RHESUS MONKEYS

Group	Control	9 Mg/kg/day	32 Mg/kg/day	64 Mg/kg/day	128 Mg/kg/day
Germinal epithelium	Unilayered with flat cuboidal cells	Unilayered with flat columnar cells	Pseudostratified with squamous cells	Pseudostratified with squamous cells	Unilayered with tall columnar cells
Average number of primary follicles	339.0	280.0	280.0	80.0	68.0
Average number of corpora lutea	3.6	14.0	30.0	20.0	30.0
Ratio of corpora lutea to primary follicles	0.01	0.05	0.1	0.25	0.44
Thickness (μm) of the ovarian germinal epithelium	7.7	12.0	14.5	16.0	21.7

increased rates of steroid removal may lead to increasing steroid production by follicles and their eventual exhaustion.

To investigate this hypothesis, a study was initiated to measure LH, FSH, progesterone, and total estrogen in rhesus monkeys through at least one menstrual cycle during treatment with HCB or PCB. We expected the results of this study to point to the site of action of the chemicals. If

(a) LH or FHS were altered, the effect would have to be on the pituitary or hypothalamus;

(b) levels of progesterone or estrogens were changed and the gonadotropins unaffected, the ovary or the liver would be likely sites of action;

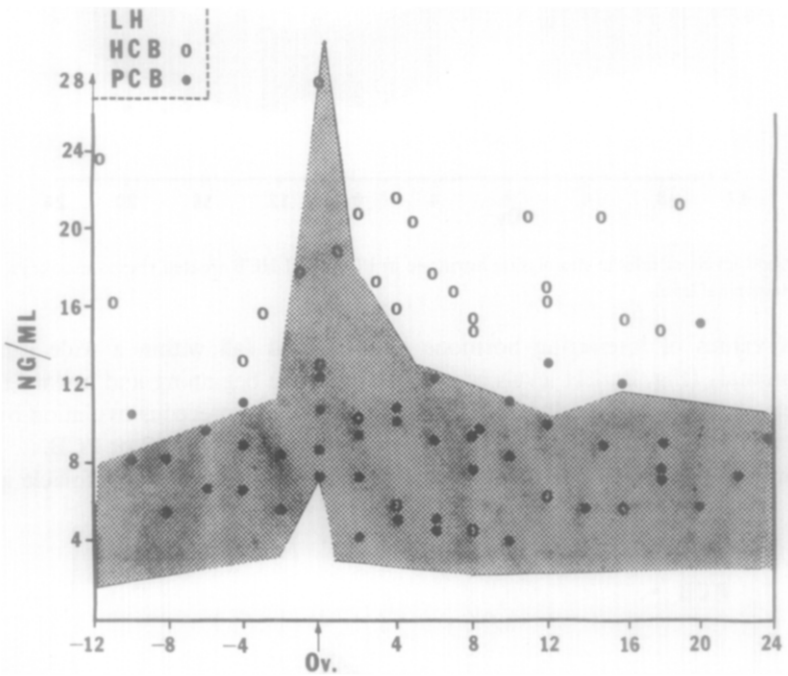

FIG. 2. Blood levels of luteinizing hormone in PCB- and HCB-treated rhesus monkeys. Shaded areas indicate normal levels.

(c) ovulation was blocked, direct poisoning of the follicles would have to be considered unless changes in gonadotropin and steroid levels were seen.

For this study, 16 mature female rhesus monkeys were divided into treatment groups of four for HCB, four for PCB, four vehicle controls, and four untreated. HCB and PCB were administered by gastric intubation in 1% methyl cellulose suspension in water at a dose rate of 4 mg/kg/day each; the vehicle control was intubated with blank methyl cellulose suspension. Serum LH, FSH, progesterone, and estrogen levels were monitored by radioimmunoassay before the beginning of dosage until ovulation was confirmed by laparoscopic inspection of the ovaries. Then treatment was started and continued through the following cycle with continued monitoring of the hormone levels. Figures 2 through 5 show the distribution of the levels of each hormone in all treated animals during the treatment cycle.

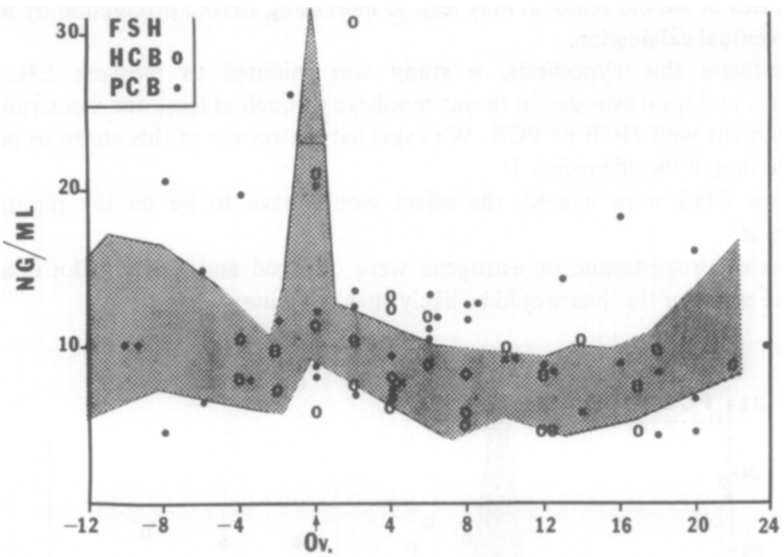

FIG. 3. Blood levels of follicle-stimulating hormone in PCB- and HCB-treated rhesus monkeys. Shaded areas indicate normal levels.

Normal values of luteinizing hormone in the blood fall within a wide range for different animals (Fig. 2). LH levels are fairly low at the beginning and toward the end of the cycle. A sharp rise produced by high estrogen levels precedes ovulation by 24 to 36 hr. No abnormal LH values are produced by treatment with HCB or PCB.

Early in the cycle, FSH levels (Fig. 3) are normally high to stimulate follicle growth.

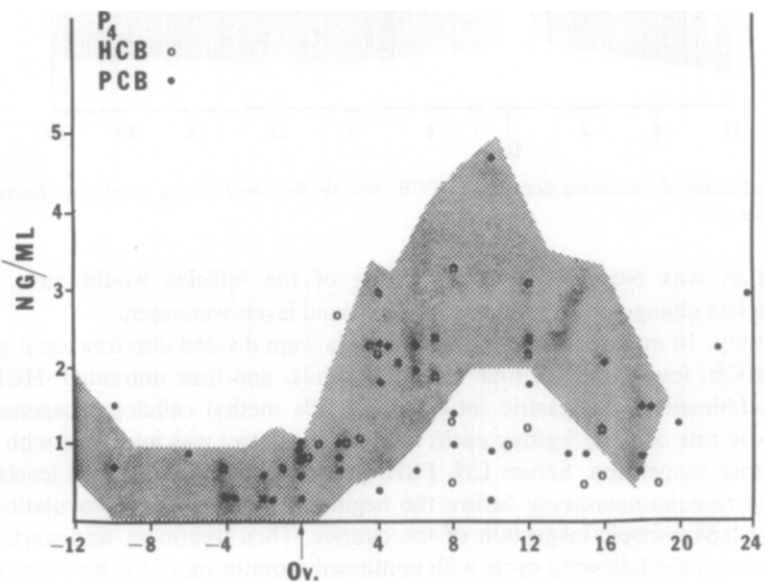

FIG. 4. Blood levels of progesterone in PCB- and HCB-treated rhesus monkeys. Shaded areas indicate normal levels.

As estrogen levels rise, FSH is suppressed. Together with the LH peak FSH is released, and during the rest of the cycle it is depressed by luteal estrogen and progesterone. Some of the treated animals show elevated FSH levels in the later phase of the cycle, indicating a lack of suppression by steroids (anovulatory cycles, no luteal steroid production).

Progesterone (P_4) levels are normally low before ovulation, begin to rise at ovulation, reach a peak 4 to 12 days later, and decline subsequently until menstruation (Fig. 4). P_4 is produced almost solely by the corpus luteum. Some of the treated animals show no P_4 rise, indicating absence of corpus luteum (anovulatory cycles).

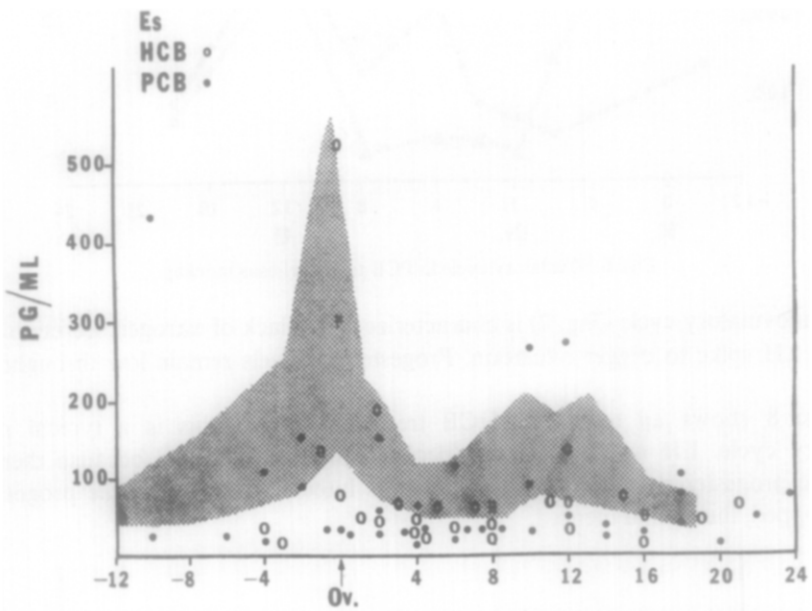

FIG. 5. Blood levels of total estrogens in PCB- and HCB-treated rhesus monkeys. Shaded areas indicate normal levels.

Most of the PCB-treated monkeys show estrogen levels below normal (Fig. 5). Lack of an estrogen peak prevents the LH surge which normally triggers ovulation. Without ovulation, there is no increase in estrogens during the luteal phase.

There was, especially in the PCB-treated group, a marked effect on ovulation. Whereas among 16 control cycles with identical bleeding schedules only one cycle was anovulatory and no double ovulations were seen by laparoscopy, of four PCB-treated monkeys two did not ovulate and one had a double ovulation. Of four HCB-treated animals, one did not ovulate during the treatment cycle.

Figures 6 and 7 show an ovulatory and an anovulatory PCB treatment cycle. In the ovulatory cycle (Fig. 6), the estrogen level prior to ovulation was high enough to cause an LH peak. Beginning 2 days after ovulation, the progesterone level rises. Twelve days after ovulation, the estrogen level rises high enough to cause a breakthrough bleeding. This is probably not a treatment effect, since it is not uncommon in rhesus monkeys.

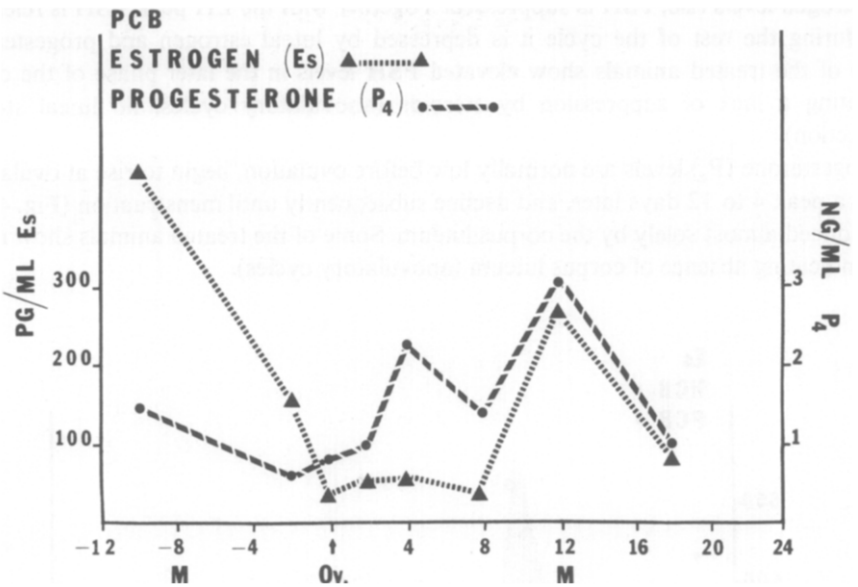

FIG. 6. Ovulatory cycle in PCB-treated rhesus monkey.

The anovulatory cycle (Fig. 7) is characterized by a lack of estrogen secretion. There was no LH spike to trigger ovulation. Progesterone levels remain low throughout the cycle.

Figure 8 shows an ovulatory HCB treatment cycle. This is a typical normal ovulatory cycle. LH and FSH levels declined after their peaks because there was adequate progesterone secretion by the corpus luteum. As soon as the progesterone level dropped, the animal mensed.

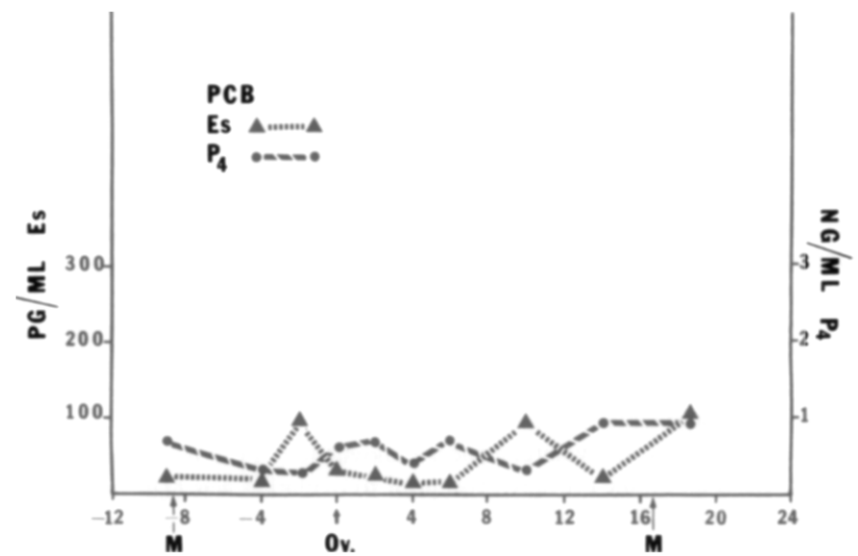

FIG. 7. Anovulatory cycle in PCB-treated rhesus monkey.

In the anovulatory cycle of one HCB-treated monkey (Fig. 9), the levels of LH and FSH continued to climb, since steroids, especially estrogens, remained low. At the end of the normal cycle length there was inadequate progesterone withdrawal, which resulted in delayed menstruation (39 days).

In each case without ovulation, there is an indication of inadequate estrogen secretion, suggesting some kind of action on the ovary. After ovulation, the luteal function proceeds normally. It may therefore be assumed, that the action of the chemicals takes place in the follicular section of the cycle, and this assumption is supported by the histopathological findings of depletion of primary follicles.

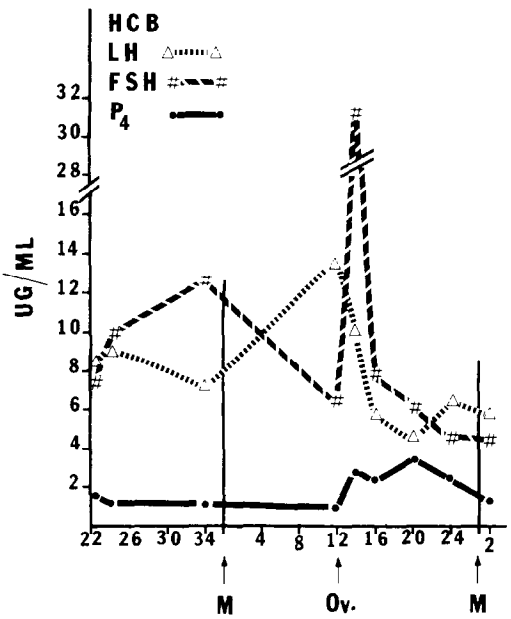

FIG. 8. Ovulatory cycle in HCB-treated rhesus monkey.

When adequate estrogen levels were present, LH spikes were seen, indicating that the positive feedback mechanism was not impaired and the function of hypothalamus and pituitary was not altered. The negative feedback also did not seem to be impaired, because as estrogen and progesterone levels rose, LH and FSH levels dropped.

The low estrogen levels we observed could indicate either a direct effect on ovarian secretion or an indirect effect through increased liver metabolism, resulting in accelerated estrogen removal.

To study the effect of HCB and PCB on ovarian steroidogenesis, the following experiment was designed.

Immature female rats were treated with pregnant mare serum at the age of 29 days to stimulate follicular development. On Day 31, the rats were treated with human chorionic gonadotropin to luteinize the follicles. On Day 34, they were given 20 mg/kg of HCB or Clophen A 30 by subcutaneous injection.

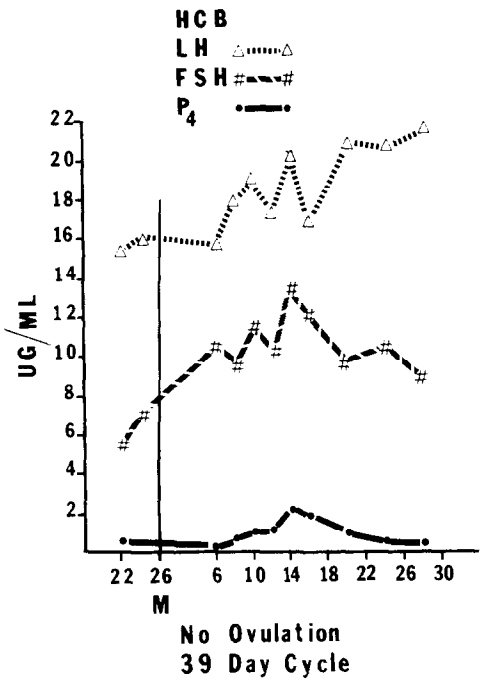

FIG. 9. Anovulatory cycle in HCB-treated rhesus monkey.

The rats were sacrificed on Day 36, and the superovulated ovaries containing a high proportion of luteal tissue were removed and finely sliced. Samples of 40 to 50 mg of tissue were placed in nutrient medium. LH was added to half of the tubes. The samples were incubated for 3 hr at 37°C, then frozen on dry ice and homogenized. Progesterone was measured by RIA.

The results, summarized in Table 3, show an increase of progesterone production in both the HCB and PCB-treated samples, especially after stimulation with LH.

Although the study with rhesus monkeys indicates that low estrogen levels rather than increased progesterone levels are causing problems, this experiment indicates some interference with ovarian steroidogenesis. The next step in this direction will be to design an experiment for studying the effect of HCB and PCB on estrogen production of ovarian tissue in the follicular phase.

TABLE 3

In Vitro P_4 SYNTHESIS AFTER *in Vivo* TREATMENT WITH PCBs OR HCB[a]

	Base synthesis	plus LH
Control	23.1 ± 2.2	27.7 ± 2.5
HCB	29.7 ± 2.8	35.8 ± 5.1
PCB	26.9 ± 3.2	47.6 ± 5.3[b]

[a] Mean ± SD.
[b] $P < 0.01$.

Experiments designed for the study of the influence of HCB and PCB on the metabolism of steroids in the liver have so far not shown very conclusive results. Although, at least for PCB, induction of cytochrome P-450-dependent metabolizing enzymes has been reported (Risebrough, 1968; Litterst, 1972; Chen, 1973), an *in vivo* study in which we injected HCB- and PCB-pretreated monkeys with radiolabeled estradiol and measured the disappearance rate of radioactivity and the estrogen metabolite patterns in blood and urine so far has failed to show a significant difference from controls.

Finally, to test for possible interaction of HCB and PCB at the pituitary level, an experiment was designed to study the pituitary response to gonadotropin releasing hormone under the influence of the chemicals. Ovariectomized rats were divided into controls and PCB- and HCB-treated groups and given GnRH with subsequent RIA measurement of LF and FSH. No significant difference in levels of these hormones was found between treated and untreated rats. In conclusion referring to our hypothesis, we can say that:

(1) There is not much evidence of HCB and PCB interaction at the hypothalamus–pituitary level;

(2) low estrogen levels in cycling rhesus treated with HCB or PCB seem to be involved in blocked ovulation;

(3) it remains to be investigated whether the reduction of estrogens is caused by a decrease in ovarian production or an acceleration of liver metabolism, or a combination of both.

ACKNOWLEDGMENTS

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