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Hormonal Alterations in Female Rhesus Monkeys Fed a Diet Containing 2, 3, 7, 8-Tetrachlorodibenzo-*p*-dioxin

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Considerable attention has been drawn to potential hazards of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Varying amounts of TCDD are produced in side reactions during the synthesis of polychlorinated phenols, 2,4,5-trichlorophenoxyacetic acid herbicide (2,4,5-T) and related compounds. Industrial accidents resulting from overheating reaction mixtures of trichlorophenol have led to explosions and release of large quantities of TCDD. The most recent accident occurred in northern Italy where an entire town was exposed to TCDD and other phenolic compounds. Soil samples from the contaminated area contained levels of TCDD in excess of 50 ppm (RAWLS and O'SULLIVAN 1976). The widespread use of 2,4,5-T has been another source of human exposure to TCDD. In Vietnam, a mixture of 2,4,5-T and 2,4-D (Agent Orange) which contained TCDD at levels as high as 49 ppm was used extensively as a defoliant. Widespread injurious effects on man and lower animals have been reported as a result of exposure to this mixture. Clinical reports from Vietnam suggest reproductive abnormalities in the human and animal population of the sprayed areas. More recently, CARTER et al. (1975) reported a high incidence of abortions in horses that lived in an arena that had been sprayed with waste products from trichlorophenol production containing high levels of TCDD. Reduced spermatogenesis has also been reported in monkeys exposed to commercial fat products adulterated with TCDD (ALLEN and CARSTENS 1967).

Since there seems to be little doubt that TCDD exposure influences reproduction, further clarification of this question was deemed important. The presently reported experiment addresses this problem through the use of nonhuman primates that have been exposed to low levels of TCDD.

MATERIALS AND METHODS

Sixteen (16) adult female rhesus monkeys (Macaca mulatta), weighing an average of 5.6 kg, were individually housed in a controlled environment simulating the light, temperature and humidity of a 20 day period of the breeding season of their native India. The animals were fed a diet containing Purina Monkey Chow (Ralston-Purina Co., St. Louis, MO) supplemented with fruit twice weekly and water ad libitum.

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For six months the menstrual cycles of these females were evaluated as to length, intensity and duration. In addition, 5 cc of blood were obtained daily via femoral venipuncture throughout an entire menstrual cycle for the determination of serum 17β-estradiol and progesterone levels (BIELERT et al. 1976). Following the period of control evaluations, the animals were divided into two groups. One group of eight animals was fed a diet to which 500 parts per trillion (ppt) TCDD had been added for nine months. The food was prepared by the addition of the proper amounts of corn oil-suspended TCDD to ground Purina chow. The TCDD-containing chow was uniformly mixed and subsequently repelletted. The other group received a similar diet devoid of TCDD. A 200 g portion of the food was given daily. Food intake was monitored in all animals throughout the nine months. Hemograms and clinical chemistries, including total serum lipid, serum cholesterol, serum glutamic pyruvic transaminase (SGPT), total serum protein and serum albumin globulin ratios were performed on a monthly basis. After 1, 3 and 6 months on the diet, blood samples were obtained throughout the menstrual cycle in order to establish serum progesterone and estradiol levels. After being on the experimental diet for 6 months, the animals were housed with control males at the appropriate time of the menstrual cycle (VALERIO 1969). Pregnancy was confirmed by obtaining a 5 ml blood sample 20 days following breeding, and serum from this blood was then used in the immature mouse bioassay for monkey chorionic gonadotropin as described by WILSON et al. (1972). Further clarification of pregnancy was obtained at 30 days by rectal palpation of the uterus.

RESULTS

The morphological changes that were produced in the TCDD exposed females have previously been reported (ALLEN et al. 1977) and will be summarized here. Within 6 months following exposure to 500 ppt of TCDD in the diet, which amount to approximately 2 μg per kg body weight, the animals became anemic. A severe pancytopenia was apparent within 9 months and after the animals had consumed approximately 3 μg of TCDD per kg body weight. Widespread hemorrhage was related to the marked thrombocytopenia. Death occurred in five of the eight animals between the seventh and twelfth month of the experiment. At necropsy, in addition to the extensive hemorrhage, there was a distinct hypocellularity of the bone marrow and lymph nodes. Hypertrophy, hyperplasia and metaplasia of the epithelium and bronchial tree, bile ducts, pancreatic ducts, salivary gland ducts and palpebral conjunctivae were observed. Squamous metaplasia and keratinization of the sebaceous glands and hair follicles were present in the skin. A severe hyperplastic gastritis with ulceration of the gastric mucosa was present in the animals that died. Death was attributed to complications arising from the severe pancytopenia.

The length of the menstrual cycles of the monkeys as well as the intensity and duration of menstruation were not altered appreciably during the initial 6 months of the experiment. The length of cycle for the experimental animals was 27.8 ± 3.9 days and the duration was 3.9 ± 1.4 days. Control animals had a cycle of 27.3 ± 4.3 days and a duration of 3.4 ± 1.3 days.

Individual pretreatment steroid patterns were used as controls for comparing estradiol and progesterone levels at 1, 3 and 6 months of the study. One animal had an unsuccessful breeding history and thus was eliminated from the steroid evaluation. The steroid analysis at 6 months showed alterations in 5 of the 7 animals. The values of the remaining 2 animals were unaltered at 6 months. Progesterone levels in 3 animals (Nos. 38, 49, 7) decreased to 72.4%, 51.9% and 47.3% of their pretreatment values, respectively. In the same time period, estradiol levels in two of these animals (7 and 49) also decreased to 50.4% and 43.2% of the control, respectively, while estradiol remained unchanged in the remaining animal (38). The additional 2 animals studied (23 and 32) revealed anovulatory patterns of both steroids. Progesterone remained below 400 pg/ml serum and estradiol never rose above 80 pg/ml serum throughout the cycles. Examples of these steroid patterns are shown in figures 1 and 2.

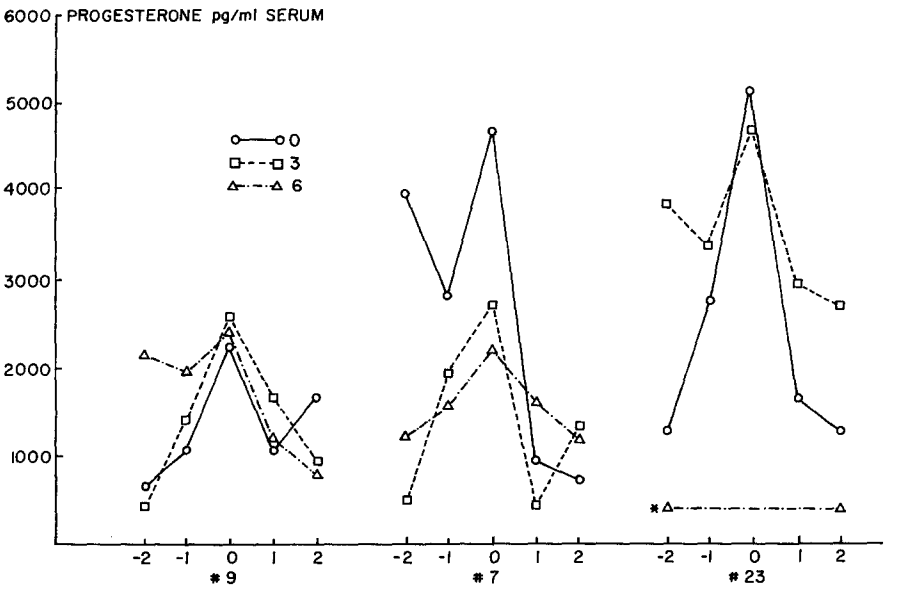

Fig. 1. Animals 9, 7 and 23 serve as examples of the radioimmunologic data on serum progesterone at 0, 3 and 6 months on the diet. The data was normalized to the progesterone peak (day 0) ± day 1 and 2. * = Anovulatory cycle. Progesterone values were below 400 pg/ml serum indicating an anovulatory cycle.

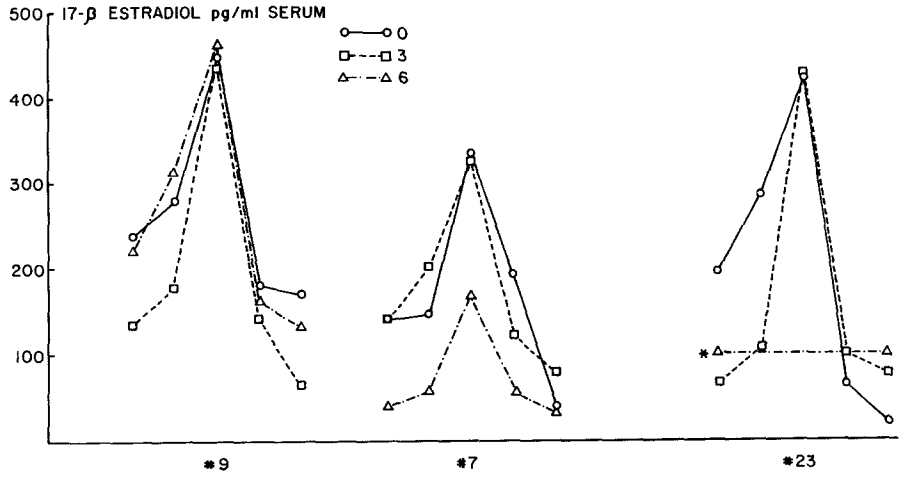

Fig. 2. Animals 9, 7 and 23 serve as examples of the radioimmunologic data on serum 17β-estradiol at 0, 3 and 6 months on the diet. The data were normalized to the preovulatory estradiol peak (day 0) ± day 1 and 2. * = 17β-estradiol values were below 80 pg/ml serum indicating an anovulatory cycle.

After the radioimmunologic evaluations were completed at the end of the sixth month, the animals were bred. All of the control animals conceived and delivered healthy infants. The two experimental animals (9 and 41) which maintained normal estradiol and progesterone levels throughout the six month period conceived. However, only animal 41 was able to carry her infant to term. Animal 9 expelled a dead, partially resorbed fetus on the 62nd day of gestation. Animal 38 which showed a decrease in progesterone levels in the serum but not estradiol also conceived, but subsequently aborted on the 46th day of gestation. Animals 7 and 49 that had decreases in both of the steroid hormones failed to conceive following repeated breedings. Such was also the case with Animal 23 which showed an anovulatory pattern as indicated by serum steroid levels. Animal 32 which also showed an anovulatory steroid level was not bred due to her debilitated state.

Following conception, Animals 9 and 38 showed intense and prolonged implantation bleeding. Menstruation became intense and sporadic in Animals 7 and 49. The loss of blood resulting from the excessive implantation bleeding and menstruation markedly decreased the hemoglobin levels of these already anemic animals.

Of the seven animals that were evaluated for their reproductive capabilities following TCDD exposure, only Animal 41 was able to carry her infant to term. Throughout the course of the experiment, this animal showed only minimal effects of TCDD exposure even though her level of consumption was equally as great or greater than that of those animals that experienced morbidity and mortality. Five of the seven animals died between the seventh and twelfth month of the experiment. The two surviving animals, 41 and 23, continued to be evaluated following the discontinuation of its TCDD diet. Animal 41 gave birth to a well developed infant. The infant remained healthy during the 4 months of nursing as determined by gross appearance and hematologic and immunologic evaluations. After the infant was weaned and a normal menstrual cycle was reestablished, the serum estradiol and progesterone were once again evaluated and found to be normal.

Animal 23 showed a decided leukopenia and thrombocytopenia after 9 months of TCDD exposure (3.8×10^3 white blood cells per mm^3 and 28×10^3 platelets per mm^3). After 8 months on a control diet, the white blood cell counts returned to normal, and the platelets increased to 364×10^3 per mm^3 blood. Within 6 months the level of serum estradiol and progesterone had also reestablished a pretreatment level. The animal was subsequently bred and gave birth to a well developed infant 5 1/2 months later.

DISCUSSION

There appears to be little doubt that the consumption of a diet containing 500 ppt of TCDD over 9 months by nonhuman primates is capable of causing reproductive abnormalities. The seven female monkeys that were included in this experiment had a history of normal menstrual cycles and had given birth to normal infants during the year prior to this study. In addition, the levels of serum estradiol and progesterone throughout the menstrual cycle of these animals were similar to those of animals of the untreated breeding colony. Thus, it can be said that prior to TCDD exposure the animals utilized in this experiment had a normal reproductive history.

Following 6 months of TCDD ingestion, during which time the food intake was similar to that of the controls, five of the seven animals showed a varying degree of modification in their serum estradiol and progesterone. Even though the total intake of TCDD was essentially the same for each animal, it was not surprising that all monkeys did not respond in a similar manner. Much like the human population, nonhuman primates are a rather heterogeneous group and considerable amounts of biological variation are to be expected.

The question arises as to how much influence the general toxic effects which include a moderate to severe pancytopenia had on the reproductive capabilities of these animals. During the period when the majority of the animals were not obviously ill, some of the animals began to show modifications in their serum estradiol and

progesterone levels. These animals also had difficulty breeding and maintaining pregnancy. Thus, it appears that the reproductive problems arose prior to the more debilitating toxic manifestations.

A possible link exists between the reproductive dysfunction associated with TCDD exposure and the increased metabolism of steroids. The fact that many chemicals increase hepatic microsomal enzymes as well as accelerate metabolism of steroids has been documented (HART and FOUTS 1963, CONNEY 1967, PEAKALL 1967, WELCH et al. 1967). TCDD administration to pregnant rats caused a marked elevation of some maternal hepatic microsomal enzymes and a unique transplacental induction of microsomal enzymes in fetal and newborn systems (LUCIER et al. 1975, BERRY et al. 1976,1977). This TCDD induction mechanism could cause an alteration in the intricate balance of steroids leading to the high incidence of fetal mortality and early and late resorptions (SPARSCHU et al. 1971) and morphologic suppression of reproductive organs in rats (KOCIBA et al. 1976) as well as the altered steroid patterns and fetal wastage reported here.

Indications are that if the TCDD exposed animals survive the toxic effects and are allowed to recover, they will once again establish a normal menstrual cycle and be capable of breeding and reproducing. In the one severely affected animal that did survive, a normal menstrual cycle as well as pretreatment levels of estradiol and progesterone were established within 6 months following the discontinuation of TCDD exposure. This animal became pregnant, experienced an uneventful gestation and gave birth to a well developed infant.

Similar reproductive abnormalities have also been reported in nonhuman primates exposed to other chlorinated aromatic hydrocarbons (ALLEN and BARSOTTI 1976, ALLEN and NORBACK 1976, BARSOTTI et al. 1976). In these experiments, the rhesus monkeys were exposed to low levels of polychlorinated biphenyls for 18 months. During this time the animals experienced menstrual irregularities and increased excretion of urinary 17-ketosteroids (BARSOTTI and ALLEN 1975). Subsequently, they had difficulties in conceiving and experienced a high incidence of early abortions. However, when the animals were removed from the experimental diet for one year, menstrual cycles and their breeding and gestational performance were similar to those of the controls.

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