

Premature Births in California Sea Lions: Association with High Organochlorine Pollutant Residue Levels

Abstract. Premature pupping in California sea lions has been noted on the breeding islands since 1968. Organochlorine pesticides and polychlorinated biphenyl residues were two to eight times higher in tissues of premature parturient females and pups than in similar tissues of full-term parturient females and pups collected on San Miguel Island in 1970.

The California sea lion (*Zalophus californianus californianus*) breeds on California's Channel Islands and on islands off the Pacific and Gulf coasts of Baja California, Mexico. In the Channel Islands normal pupping occurs between 15 May and 30 June and breeding between 20 June and 20 July. We have observed early termination of pregnancies among animals on several breeding rookeries, including those on San Miguel Island, since 1968. Premature births were reported by Simpson and Gilmarin (1) on San Miguel Island and by Odell (2) on San Nicolas Island, and were discussed by Brownell and LeBoeuf (3). An earlier investigation into the causes of these premature births was inconclusive (1). The work reported here was conducted to determine whether environmental pollutant residues may be related to premature parturition in the California sea lion.

In the Channel Islands premature births occur as early as January and appear with increasing frequency until mid-May. We observed live premature pups from February through mid-May, and saw no pups which were dead at birth. The early pups are not furred and appear to die soon after birth. The premature pups born in March, April, and early May are furred, but they lack motor coordination, their breathing is short, and only portions of the lungs have been aerated. Late premature pups appear to live from a few hours to possibly as long as several days. There is no apparent temporal break between

the end of premature births and the onset of the normal pupping season. About 15 May the death rate decreases and some pups survive. In mid-May both live premature and full-term pups are present; full-term pups are easily distinguished as they have full motor coordination. The quantitative incidence of premature births has not been well established. On San Miguel Island we counted 242 dead premature pups on 25 April 1970 and 348 on 18 May 1971 from an estimated total population of 10,000 to 15,000. On San Nicolas Island, Odell (2) reported 442 premature pups from a female population of 5,500 between 17 January and 3 May 1970.

In 1970 we collected two groups of postpartum female California sea lions within 24 hours after parturition on San Miguel Island. The premature parturient group of six females and their premature pups were collected between 20 and 24 April. The full-term parturient group of four females and their live pups were collected between 24 and 26 June, late in the normal pupping season.

Ages of adult females were determined by counting growth layers in the dentine of longitudinally sectioned canines (4). The liver, brain, and blubber from cows and pups were analyzed for DDT compounds (*p,p'*-DDT, *p,p'*-DDD, and *p,p'*-DDE), dieldrin, and polychlorinated biphenyls (PCBs) (5). Identification of PCB's was based on the characteristic profiles of

peaks on the gas chromatogram obtained with a DC-200 column; quantification was achieved through a comparison of the height of the peak eluting between the peaks for *p,p'* and *p,p'*-DDT with the height of the corresponding peak in chromatograms of the standard (Aroclor 1254, Santa Co.) (6). Blubber and liver from females were analyzed as representative storage and metabolizing tissues for organochlorine residues. Brain tissue from pups was analyzed to some insight into the behavior of premature pups. Organochlorine pesticide and PCB residues were found in all tissues collected and tested (1). The most prevalent metabolite found to be DDE (80 to 93 percent the total DDT).

All analyzed tissues from females which gave birth prematurely contained higher concentrations of the residues from females which birthed to full-term pups. The mean DDT residues in the blubber and of premature parturient females 8.0 and 3.8 times greater, respectively than the concentrations in the tissues of normal parturient cows. Brains of premature pups contained twice the residue concentrations as brains of full-term pups. The levels in blubber, liver, and brain of premature parturient group were 4.4, and 2.4 times higher than in the full-term group. In the blubber nor liver did the ranges of due values of total DDT or PCBs in premature and full-term groups overlap (Table 1). Dieldrin residues not detected in all samples and present, were low. Figure 1 is a histogram of the DDT and PCB residues of the two groups of animals, showing relative differences in tissue pollutant concentrations.

Histological sections of liver tissue revealed normal tissues from all pre-

Table 1. Concentration of pollutant residues in tissues of parturient California sea lion females and pups, in parts per million (ppm), weight; N, number of animals; X, mean of two (brain, liver) and three (blubber) residue determinations on each tissue of each animal. The age range is 6 to 12 years for premature parturient females and 10 to 15 years for full-term parturient females. The numbers in parentheses are standard deviations.

Tissue	N	Mean age (years)	Fat (%)		DDT (total)		PCBs	
			Mean	Range	$\bar{X}$ (ppm)	Range (ppm)	$\bar{X}$ (ppm)	Ra (ppm)
Premature								
Blubber (female)	6	8	84	77-87	824.4 (167.5)	626-1039	112.4 (21.4)	5.5
Liver (female)	3	8	1.5-2.2	2.3-4.7	25.24 (4.1)	22-30	5.74 (1.45)	3.4
Brain (pup)	6		3.3	3.2-3.4	2.38 (1.27)	0.82-4.15	0.45 (0.32)	0.23
Full-term								
Blubber (female)	4	12	85	84-88	103.2 (69.7)	51-203	17.1 (6.1)	12
Liver (female)	4	12	4.4	2.9-5.5	6.67 (5.1)	2.4-13.6	1.32 (0.76)	0.52
Brain (pup)	4		4.8	4.6-5.1	1.20 (1.10)	0.36-2.82	0.19 (0.05)	0.12

nure partus females except one. The liver of this animal showed a few mid-axial foci of mononuclear and polymorphonuclear cell infiltrates. These abnormal findings apparently are not similar to histological changes in rats fed organochlorine pesticides (7) or rodents fed PCB compounds (8). Serological analysis revealed no antibody titers to *Leptospira* species or *Brucella abortus* in the six cows of the premature parturient group. Bacteriological analysis of the uteri of this group yielded isolation of *Escherichia coli* from two of the six.

Liver mercury residues (wet weight) in the three premature parturient females ranged from 38 to 64 parts per million (ppm) and in their pups ranged from 0.4 to 1.8 ppm. None of the tissues from full-term animals were analyzed for mercury.

The difference between the concentrations of organochlorine pesticide and PCB residues in the premature and full-term groups indicates a possible cause and effect relationship between high levels of these pollutants and early termination of pregnancy in California sea lions. High concentrations of organochlorine residues are implicated in reproductive failure in ranch mink fed fish from Lake Michigan (9). Experimental feeding of PCB compounds has been shown to cause reproductive failure and death in adult ranch mink (10). There is also experimental evidence that *p,p'*-DDT induces premature parturition in rabbits (11). Some organochlorine insecticides and PCB compounds induce hepatic microsomal enzyme activity resulting in increased metabolism of progesterone and estradiol in pigeon livers (12). Similar enzyme induction has been demonstrated in the rat (7). Definitive knowledge of the combined toxic or synergistic effects of organochlorine pesticides, PCBs, and mercury is lacking. LaBoeuf and Bonnell (13) reported high residue levels from three "apparently healthy" female California sea lions collected in September 1970 at San Miguel Island. Apparent good health of the female does not exclude a history of premature parturition. Since they did not report the reproductive histories of those animals, it is impossible to relate their findings to the premature parturition phenomena.

The exact relationship of pollutants to premature births and the mechanisms involved can only be elucidated through laboratory experiments. Such experiments with California sea lions

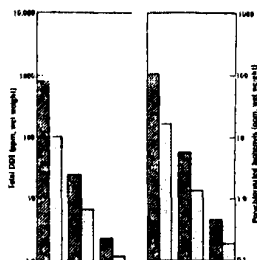


Fig. 1. Histogram of mean concentrations of total DDT and PCB's in tissues of California sea lions delivering prematurely (cross-hatching) and at full term (stippling).

have not been possible because of the cost of maintaining large breeding colonies of sea lions in captivity. However, considering the magnitude of the differences in residue levels found in the two groups sampled from the wild population, we feel that the possible cause and effect relationship cannot be ignored.

A possible explanation for the difference in pollutant residue levels in the tissues from the two groups of sea lions is utilization of different feeding areas. It has long been known that after the breeding season male California sea lions move northward. It is believed by some that many females of the Channel Islands population move south and winter in waters off southern Baja California (14), while a portion remain in the general area of the Channel Islands.

Organochlorine residues in a marine crab (15), marine fish (16), and the brown pelican (17) are higher around Los Angeles, California, than in Baja California or in northern California. Thus, female sea lions wintering in the southern areas would be feeding on fish having smaller amounts of organochlorine pesticides and would therefore assimilate smaller quantities of the pesticides than females wintering in the Channel Islands area.

The mean age of premature parturient females is less than that of full-term females (Table 1), but the age ranges of the groups overlap. Because of the small sample sizes and the overlap in age ranges it is not possible at this time to relate the observed difference in organochlorine residues or the premature parturition phenomena to age.

We think it is unlikely that the ob-

served difference in organochlorine residue concentrations could be accounted for by the premature parturient females being collected 2 months before the full-term females. Five sea lion females collected randomly from the San Miguel Island population in July 1969 had total DDT metabolite residues in the blubber ranging from 17 to 988 ppm (18), which indicates that some females can be expected to have high residues at any time of year and that a profile of the population probably ranges from animals with low to those with very high concentrations of organochlorine residues. Unfortunately, nothing is known about the fat dynamics in gestating female sea lions or about changes in organochlorine residue storage dependent on hormone cycles. Net mobilization of fat to meet increasing energy demands would be expected to increase residue levels in the blubber, yet we found that the full-term females had lower organochlorine residue concentrations.

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5. The DDT compounds were *p,p'*-DDT (1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane), *p,p'*-DDD (1,1-dichloro-2,2-bis(4-chlorophenyl)ethane), and *p,p'*-DDE (1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene). The analyses for these compounds, dieldrin, and PCB's were made by the Wisconsin Alumni Research Foundation, Madison, according to the standardized techniques given in: Food and Drug Administration, *Pesticide Analytical Manual* (Department of Health, Education, and Welfare, Washington, D.C., 1965-1968), vol. 1, loose-leaf.
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19. We thank A. M. Johnson and R. W. Riebschroug for aiding in the design and interpretation of the study and B. Anouette, M. Dailey, S. Leatherwood, and B. Strence for assistance in capturing the sea lions.
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16 October 1972; revised 30 January 1973

Cell-Mediated Immune Responses in vitro: Interaction of Thymus-Derived Cells during Cytotoxic Allograft Responses in vitro

Abstract. A mouse in vitro allograft system was used to check for synergism among thymus-derived cells during cell-mediated immune responses. Synergistic interactions occurred between thymus and thymus-derived lymphocytes. The majority of precursors of cytotoxic effector cells were peripheral thymus-derived cells; thymocytes acted mainly as amplifier cells.

Both murine thymocytes and thymus-derived (T) lymphocytes have been shown to be capable of mounting cytotoxic allograft responses in vivo (1) and in vitro (2), apparently without requiring collaboration with bone marrow (B)-derived lymphocytes. When compared on a cell to cell basis, thymocytes were found to be poor precursor cells of cytotoxic lymphocytes (CL) in vitro in contrast to recirculating T cells, splenic T cells being intermediate (2). These findings suggested that there exists a heterogeneity within thymus-processed lymphocytes in their capacity to differentiate in vitro into cytotoxic effector cells. Indeed evidence for

the existence of functional T cell subpopulations has appeared (3, 4). For example, Cantor and Asofsky (4) proposed a model for the generation of thymus-derived effector cells mediating graft-versus-host reactions, in which two distinct types of T cells appear to be involved: one acting as an amplifier cell and the other type functioning as the precursor of the effector cell. I have now tested the concept of T-T cell interactions during cell-mediated immune responses in an in vitro allograft system.

About 15×10^4 viable dissociated CBA/H/Wehi (H-2^b) or AKR/J (H-2^k) mouse thymocytes or inguinal

lymph node (LN) cells, or a mixture of both, were cultured together 2×10^6 mitomycin C-treated B. spleen cells (H-2^d) for 6 days in fied Eagle's medium containing 10% fetal calf serum in the Mar Diener culture system (2, 5), from three cultures per group pooled, washed twice, and assayed in vitro generated cytotoxic activity a modification (2) of the ^{51}Cr release assay described by Brunner *et al.* Cytotoxic activity generated in vitro compared on a culture basis (7). The number of lymphocytes at the start of the culture was kept identical in the various groups of cultures the cytotoxic activity generated compared relative to that obtained LN cells as precursor cells of cytotoxic lymphocytes. In actual fact, lymphocytes harvested from different groups were assayed for cytotoxicity at the cell concentration + resulted (in the case of LN derived cytotoxic lymphocytes) in a ratio cytotoxic lymphocytes to target of 12 to 1, 2 to 1, and 0.3 to 1. DBA/2 derived mastocytoma cell P815 (8), cultured in vitro on identical H-2 specificity as BALB/c spleen cells used for immunization, used as target cells in the ^{51}Cr release assay. AKR antiserum to CBA θ antigen was prepared according to (9) and used as described (7). Antiserum killed 80 percent of thoracic duct lymphocytes in the presence of agarose-adsorbed guinea serum, and the activity against θ cells was absorbed by brain from CBA θ Mitomycin C treatment was administered at a final concentration of $\mu\text{g/ml}$ for 30 minutes (2). Irradiation (900 rads) of dispersed lymphocytes was performed as described by M and Sprent (10).

As given in Table 1, CBA LN cells (an example of a lymphocyte population containing 80 percent of peripheral T cells) represented a good precursor cell population for cytotoxic lymphocytes. Thus, after a 6-day culture period in the presence of cell-bound θ antigen (2×10^6 mitomycin C-treated BALB/c spleen cells), cytotoxic activity was generated which lysed 80 percent of the ^{51}Cr -labeled P815 target cells at a ratio of cytotoxic lymphocytes to target cells of 2 to 1 within 200 minutes. That the reacting cells were lymphocytes was suggested by the fact that treatment of LN cells before culture with AKR antiserum to θ antigen plus complement, abolished the capacity of the cells to mount a cytotoxic

Table 1. Synergistic effect of a mixture of thymocytes plus syngeneic lymph node cells in cytotoxic allograft responses in vitro.

Test system	Lysis (%) ^a of ^{51}Cr -labeled P815 target cells (H-2 ^d) at CL to target cells ratio of:		
	12:1	2:1	0.3:1
15×10^4 CBA LN	100	80	40
15×10^4 CBA LN, treated with antiserum to θ	13	12	12
15×10^4 CBA thymocytes	30	14	12
1.5×10^4 CBA LN plus 14×10^4 CBA thymocytes	25	13	12
1.5×10^4 CBA LN plus 14×10^4 CBA thymocytes	100	91	52
1.5×10^4 (CBA LN), treated with antiserum to θ plus 14×10^4 CBA thymocytes	25	14	12
1.5×10^4 (CBA $\times$ BALB/c) F ₁ LN plus 14×10^4 CBA thymocytes	23	13	12
1.5×10^4 CBA LN plus 14×10^4 (CBA $\times$ BALB/c) F ₁ thymocytes	38	27	13
1.5×10^4 (CBA LN) mitomycin C-treated plus 14×10^4 CBA thymocytes	24	12	12

^a Responder cells (H-2^d) were cultured together with 2×10^6 mitomycin C-treated allogeneic BALB/c (H-2^k) spleen cells. Cytotoxic activity generated in vitro was assayed in a ^{51}Cr test for 200 minutes. Cytotoxicity obtained was compared on a culture basis relative to the cytotoxicity generated by CBA LN cell derived cytotoxic lymphocytes. Background lysis of P815 target cells (in the presence of normal lymphocytes) was 17 ± 1.3 percent. Similar results were obtained in 12 independent experiments.