Lymphocyte Function of Michigan Dairy Farmers Exposed to Polybrominated Biphenyls

Abstract. *Michigan dairy farm residents ate farm products containing polybrominated biphenyls (PBB's) after the accidental contamination of animal feed with the chemical in that state in 1973. The circulating blood lymphocytes of these residents show significant changes. Abnormalities include decreases in the numbers and percentages of peripheral blood lymphocytes that form rosettes with either sheep erythrocytes alone or with sheep erythrocytes sensitized with antibody and complement, increases in lymphocytes with no detectable surface markers ("null" cells), and altered responses to tests designed to evaluate functional integrity of the cells. There appears to be no consistent correlation between the concentration of PBB's in the plasma and the altered lymphocytes. Studies showed that in Wisconsin dairy farm residents and healthy individuals in the New York area who were not exposed to PBB's there were no such abnormalities.*

In 1973, polybrominated biphenyls (PBB's) were accidentally used in place of magnesium oxide in the preparation of a special feed supplement for lactating cows (*1*). Subsequently, toxic effects were observed in animals given PBB-contaminated feed. These effects included decreased milk production, swelling of the joints, hyperkeratosis, persistent mastitis, cutaneous and subcutaneous infections including abscess formation on the back, legs, and udder, and slow wasting and death. These phenomena were seen within 6 months in 24 of one group of 400 exposed cows (*2*). More than 500 Michigan dairy herds and poultry farms were quarantined. Over 34,000 cattle and 1.5 million chickens died or were destroyed, as well as smaller numbers of swine and other farm animals. During this period, meat and dairy products containing PBB's (beef, poultry, pork, eggs, milk, cheese) were widely consumed in Michigan. Polybrominated biphenyls were subsequently found in the serum and adipose tissues of dairy farmers in the state as well as in many urban Michigan residents (*3*). Polybrominated biphenyls are known to be stored in adipose tissues and to persist there for very long periods. Moreover, like polychlorinated biphenyls (PCB's), these materials accumulate in the tissues (*4*).

During a clinical field survey of PBB-exposed persons (*5*) a small group of Michigan dairy farm residents exposed to PBB's was studied by various immunodiagnostic methods. Since these tests showed significant deviation from normal values, 45 adult Michigan dairy farmers and members of their families who had been examined in the field survey were invited for further study of their immunological status (*6*). They had eaten PBB-contaminated food for 3 months to 4 years after the original accidental contamination of cattle feed and many considered their overall health status to have significantly changed in the previous 3 to 4 years. For comparison, a similar group of 46 dairy farmers and members of their families in central Wisconsin who had not eaten PBB-contaminated food was examined, as were 79 healthy subjects in the New York area (*7*).

Peripheral blood (50 ml) was taken by venipuncture in a plastic syringe containing heparin (10 units of preservative-free heparin per milliliter of blood). Care was taken in handling the blood samples, and they were hand-carried during the journeys from Michigan to New York or Wisconsin to New York (*8*). The lymphocytes were separated 6 to 10 hours after the blood was drawn for immunodiagnostic tests in vitro. At the same time, complete blood counts, sequential multiple analyses (6 and 12) were done, and immunoglobulins M, A, and G were measured (*9*).

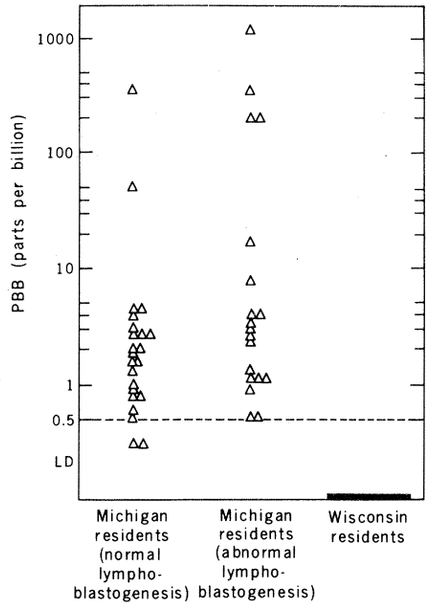

Fig. 1. Concentrations of PBB's in the serums of Michigan and Wisconsin farm residents. A hexane ether extract of methanol-treated serum was eluted through a Florisil column (Floridin) and topped with sodium sulfate. The hexane eluate was analyzed by electron-capture gas chromatography (^{63}Ni detector) with a Perkin-Elmer 900 instrument with 1 percent OV101 (3 feet by 0.25 inch) on a 80/100 Supelco glass column at 240°C. Analysis for PBB's was based on the major peak of 2,4,5-2′,4′,5′-hexabromobiphenyl. Limit of detectability (*LD*) was a total injection of 5 pg, and recovery from serum samples was satisfactory at ≥0.2 part per billion (ppb). In this figure a serum level below 0.5 ppb is designated as a trace quantity.

Table 1 summarizes phytohemagglutinin (PHA)- and pokeweed mitogen (PWM)–induced lymphoblastogenesis and mixed leukocyte culture values for the PBB-exposed Michigan dairy farm residents and the two control populations. Cultures were made with both autologous and normal pooled AB plasmas. Since there was no quantitative difference in the blastogenic transformation of peripheral blood lymphocytes in either plasma, all data reported here refer to maximum stimulation in AB plasma. Compared to the values obtained for the two control groups, we recognized two populations among the tested Michigan residents. In 27 of 45 PBB-exposed Michigan residents the peripheral blood lymphocytes responded within a normal range to PHA and PWM, but showed reduced reactivity in mixed leukocyte cultures (Table 1). In the remaining 18 of the 45 PBB-exposed subjects the peripheral blood lymphocytes showed a significantly reduced response to PHA and PWM, and reduced reactivity in mixed leukocyte cultures. Values for maximum stimulation as measured by the uptake of [^{3}H]thymidine were one-third to one-fourth of the values of normal controls ($P < .00001$).

Table 2 shows the number and percentages of the various subpopulations of peripheral blood lymphocytes measured by two assays of rosette formation, one for sheep erythrocyte (E) rosettes and the other for sheep erythrocytes sensitized with antibody and complement (EAC) rosettes. Values obtained for the Wisconsin dairy farm residents and healthy subjects in New York were similar in each of the lymphocyte surface marker characteristics. On the other hand, for the 27 Michigan farm residents within the normal range of mitogenic response and who showed normal values for absolute numbers of E and EAC rosette-forming lymphocytes, the percentage of E and EAC rosettes was decreased and there was an increase in the number of lymphocytes without detectable surface markers ("null" cells). Eighteen of the PBB-exposed Michigan residents showed significantly reduced E and EAC rosette-forming

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lymphocytes. The surface membrane immunoglobulins as quantitated by direct immunofluorescence assay correlated well with the EAC values, 15.8 ± 1.4 percent as opposed to 17.3 ± 1.6 percent. Despite the marked changes in characteristic cell surface markers detected in the peripheral blood lymphocytes of the PBB-exposed Michigan residents, the marker for monocytes as quantitated by peroxidase staining (*10*) did not differ from that of the control population. Thus, the most significant deviation from normal values among these individuals was the marked increase in lymphocytes with no detectable surface markers.

Figure 1 shows the serum PBB values for the individuals examined. All but two PBB-exposed Michigan dairy farm residents had trace or greater (≥ 0.5 part per billion) quantities of PBB detectable in their serums. None of the Wisconsin farm residents had any measurable PBB in their serums (*11*). Although those with higher PBB concentrations had a somewhat greater tendency to have abnormal lymphocytes, there was no consistent correlation between PBB plasma concentrations and lymphocyte function in either of the two groups of PBB-exposed Michigan dairy farm residents. It is recognized, however, that PBB serum levels bear no constant relationship to fat or other tissue PBB concentrations, either in human or animal studies (*1, 3*).

Table 1. Peripheral blood lymphocyte function in PBB-exposed Michigan dairy farm residents. Lymphocyte blastogenesis for the peripheral blood lymphocytes (PBL's) was determined by selected mitogens: phytohemagglutinin (PHA) and pokeweed mitogen (PWM). After isolation of PBL's by the Ficoll-Hypaque gradient method, 100,000 PBL's were cultured in each of the five replicate wells of Falcon microplates with RPMI 1640 medium supplemented with 20 percent heat-inactivated autologous or pooled AB plasma in the presence of high-purity PHA (Burroughs Wellcome) or PWM (Gibco). Maximum stimulation occurred at 0.15 μg per well for PHA and 30 μg per well for PWM. Control cultures were incubated without mitogens in five replicate wells at 37°C in a humidified atmosphere containing 5 percent CO_2 in air. Lymphocyte blastogenesis was determined by measuring the level of DNA synthesis upon addition of 1 μCi of [^{3}H]thymidine (New England Nuclear) to each well 18 hours prior to termination of the culture. After 90 hours, the cells were harvested with a Mash II automatic harvester and the amount of [^{3}H]thymidine incorporated was determined in a Packard liquid scintillation spectrometer. One-way mixed leukocyte cultures were performed as follows: stimulation PBL's were incubated with mitomycin C (25 μg/ml of 2 × 10^6 cells in suspension) for 20 minutes at 37°C; the cells were washed, then 2 × 10^5 cells were distributed in each of the replicate wells of the Falcon microplates containing 10^5 responding lymphocytes per well in RPMI 1640 medium supplemented with 20 percent heat-inactivated autologous or AB plasma. After 90 hours of incubation, 1 μCi of [^{3}H]thymidine was added to each well. Cultures were harvested 18 hours later with the addition of an excess of cold thymidine. The data are means ± standard error. The stimulation index (SI) is calculated from the number of lymphocytes undergoing maximum stimulation divided by the number of unstimulated lymphocytes.

Subjects	*N*	Phytohemagglutinin		Pokeweed mitogen		Mixed leukocyte culture	
		Maximum stimulation (count/min)	SI	Maximum stimulation (count/min)	SI	Maximum stimulation (count/min)	SI
Normal subjects (New York)	79	102,226 ± 8,720	257	95,130 ± 6,369	194		
Wisconsin dairy farm residents	46	97,662 ± 2,693	292	90,636 ± 3,406	190	38,172 ± 1,209	66
PBB-Exposed Michigan dairy farm residents							
Demonstrating normal lymphoblastogenesis	27	92,272 ± 2,241	281	93,199 ± 7,412	186	28,248 ± 2,072*	49
With abnormal lymphoblastogenesis	18	28,457 ± 3,406*	71	39,159 ± 3,537*	77	10,147 ± 317*	20

*Statistical significance (Student's *t*-test) between maximum blastogenesis of Wisconsin farm residents and that of Michigan farm residents ($P < .00001$).

Table 2. Membrane markers of peripheral blood lymphocytes in PBB-exposed Michigan dairy farm residents. Quantification of E and EAC rosette-forming PBL's were carried out by the methods of Wybran and Fudenberg (*12*) and Nussenzweig (*13*), respectively. Sheep red blood cells were obtained from a previously tested single animal source and were stored in Alsever's solution at 4°C for no more than 6 days. Lymphocytes with three or more E or EAC rosettes were considered positive. All lymphocytes were counted to determine the percentage of total E or EAC rosettes. The absolute number of total E and EAC rosette-forming cells were determined by calculations of the corresponding total and differential leukocyte counts. In most cases the direct immunoflourescence assay for quantification of surface membrane immunoglobulin was performed as was described in Preud'homme and Seligmann (*14*). We have calculated the percentage and the number of PBL's without characteristic membrane markers by subtraction: the percentage or total number of mononuclear cells minus the percentage or absolute number of E plus EAC rosette-forming lymphocytes. Data are means ± standard error. Probabilities indicate statistical significance between percentage or absolute number of E and EAC rosette-forming lymphocytes or PBL's with no detectable surface markers from Wisconsin residents compared with Michigan residents.

Subjects	Total mononuclear cells (number/mm)	E rosettes*		EAC rosettes†		Lymphocytes with no detectable membrane markers‡	
		Percentage	Absolute number	Percentage	Absolute number	Percentage	Absolute number
Normal subjects (New York)	2589 ± 380	74.4 ± 2.4	1986 ± 251	22.1 ± 1.2	521 ± 52	3.5 ± 0.1	82 ± 15
Wisconsin dairy farm residents	2103 ± 149	71.0 ± 2.1	1473 ± 63	23.1 ± 1.1	487 ± 29	6.8 ± 0.5	143 ± 11
PBB-exposed Michigan dairy farm residents							
Demonstrating normal lymphoblastogenesis	2214 ± 162	60.0 ± 1.8 (*P* .01)	1341 ± 79	21.4 ± 1.3	467 ± 34	18.6 ± 1.2 (*P* .001)	406 ± 32 (*P* .001)
With abnormal lymphoblastogenesis	2098 ± 150	43.7 ± 4.1 (*P* <.00001)	917 ± 119 (*P* .004)	15.8 ± 1.4 (*P* .002)	331 ± 32 (*P* .004)	40.5 ± 3.9 (*P* .00001)	850 ± 56 (*P* <.00001)

*Spontaneous E rosette-forming PBL's at 4°C with sheep red blood cells. †Binding of PBL's with EAC. ‡PBL's without detectable membrane markers ("null" cells).

Although these lymphocyte changes are not found in healthy normal subjects, their short- or long-term influence on the health of the Michigan dairy farmers who were exposed to PBB's has not been established. Nor is it known what influence these alterations and associated metabolic changes will have on other individuals who have consumed PBB-contaminated food and who now bear PBB burdens.

J. GEORGE BEKESI
JAMES F. HOLLAND
Department of Neoplastic Diseases, Mount Sinai School of Medicine of the City University of New York, New York 10029
HENRY A. ANDERSON
ALF S. FISCHBEIN
WILLIAM ROM, MARY S. WOLFF
IRVING J. SELIKOFF
Environmental Sciences Laboratory, Mount Sinai School of Medicine

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4. G. F. Fries and G. S. Marrow, *Dairy Sci.* **58**, 945 (1975); R. K. Ringer and D. Polin, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **36**, 1894 (1977).
5. A clinical field study of 1040 Michigan dairy farm residents in Grand Rapids was carried out on 4 to 11 November 1976.
6. Michigan dairy farm residents who lived within 50 miles of Reed City were selected for immunological study on 17 to 18 January 1977.
7. On 26 to 28 March 1977, Wisconsin dairy farmers underwent identical clinical and laboratory examinations.
8. Control blood samples obtained from New York scientific research personnel were sent with the study samples from both Wisconsin (26 to 28 March 1977) and Michigan (4 to 11 November 1976 and 17 to 18 January 1977) to ascertain whether the plane journey might in some way cause abnormalities. None were seen.
9. Not a single individual in either group differed from the established laboratory normal values for hemoglobin, white blood cells, differential count, percentage of lymphocytes, or immunoglobulins M, A and G.
10. J. L. Preud'homme and G. Flandrin, *J. Immunol.* **113**, 1650 (1973).
11. The serum on one individual in the Wisconsin group was at the limit of detectability for the analytical methodology used (0.5 ppb).
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Pyrazole-Induced Thyroid Necrosis: A Distinct Organ Lesion

Abstract. *One oral dose of pyrazole caused necrosis of rat thyroid follicular epithelial cells but spared the parafollicular (C) cells and the parathyroid glands. Serum thyroxine (T_4) and triiodothyronine (T_3) were significantly decreased on day 3 after pyrazole administration and were immeasurable on day 5. At day 5 the thyroid was enlarged and the concentration of thyroid-stimulating hormone in the serum was increased, indicating an appropriate pituitary response to a primary lesion in the thyroid. Doses of pyrazole which produced no morphologic change in the thyroids also significantly depressed the concentrations of T_4 and T_3 in the serum.*

Endocrine glands, unlike the liver, kidney, or lung, are rarely the target of chemically induced lesions. The experimental diabetes (damage to the islet cells of the pancreas) produced by alloxan or streptozotocin (*1*), the parathyroid necrosis induced by asparaginase (*2*), the hexadimethrine bromide–caused necrosis of the pituitary gland and adrenal cortex (*3*), as well as the hemorrhage or necrosis in the adrenal gland following the administration of 7,12-dimethylbenz(*a*)anthracene (*4*), acrylonitrile (*5*), or thioguanine (*6*) are examples of the few rare endocrine lesions related to chemicals. Although extensive studies have been undertaken on the relations between structure and activity of antithyroid drugs (that is, inhibitors in various steps of thyroid hormone synthesis) (*7*) and on the goitrogenic action of chemicals (*8*) only radioactive iodine has been available to induce necrosis in the thyroid gland.

During structure-activity studies with chemicals inducing adrenal necrosis or duodenal ulcer, or both, in the rat (*9*) we found that pyrazole consistently produces structural and functional alterations in the thyroid gland. Pyrazole is an inhibitor of hepatic alcohol dehydrogenase, and hence it has been extensively used in biochemical research (*10*).

Sprague-Dawley derived Charles River CD female rats (200 g) were given unlimited access to Purina Lab Chow and tap water. They each received one dose of 30, 70, 100, or 140 mg of pyrazole

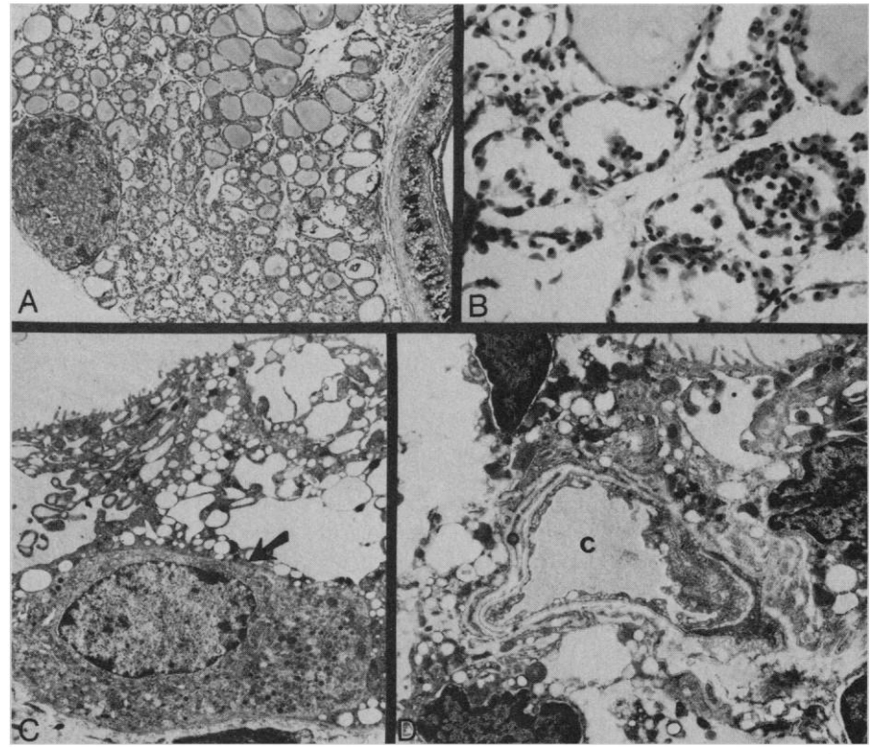

Fig. 1. (A) Extensive damage in the thyroid: only a few follicles are intact (gray). Most of the follicular cells are desquamated, the colloid is in various stages of dissolution, and the follicles appear empty (white) (×50). (B) Higher magnification of the damaged thyroid. The colloid is not visible and the desquamated follicular epithelial cells (with dark pycnotic nuclei) are aggregated. On top, two follicles are only partially involved (×280). (C) Electron micrograph of the thyroid of a rat given one dose of pyrazole (140 mg/100 g). The follicular epithelium shows marked injury (for example, dilation and vesiculation of endoplasmic reticulum) while a parafollicular C cell (arrow) appears to be intact (×6500). (D) Advanced destruction and desquamation of the follicular epithelium are evident (×8900). Notice the normal capillary (*C*).

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