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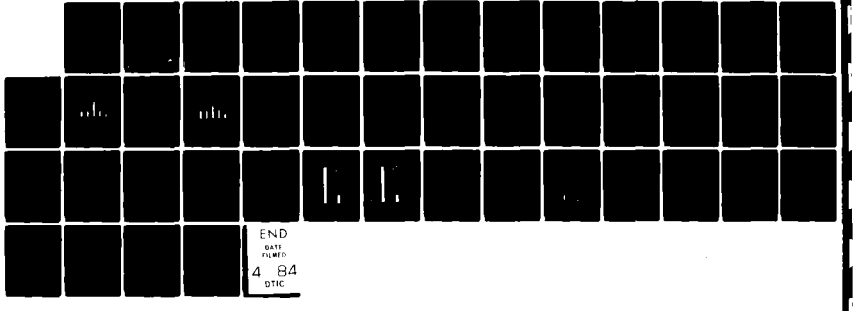

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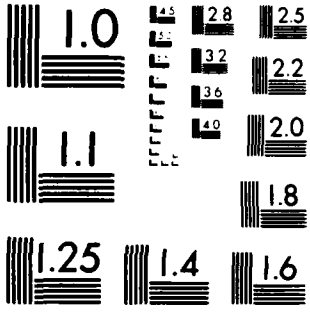

Report USAFSAM-TR-83-50

EFFECTS OF LONG-TERM LOW-LEVEL RADIOFREQUENCY RADIATION EXPOSURE ON RATS

VOLUME 5. EVALUATION OF THE IMMUNE SYSTEM'S RESPONSE

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NOTICES

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The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources - National Research Council.

The Office of Public Affairs has reviewed this report, and it is releasable to the National Technical Information Service, where it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.

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The immune system's response was evaluated in two groups of 10 rats each exposed to 2450-MHz 480-uW/cm^2 RFR in circularly polarized waveguides for 21 h per day for 13 (Group 1) and 25 months (Group 2); two other groups of 10 rats each served as controls. Increases in the numbers of B- and T-cells and enhancement of the lymphocytic response to mitogen stimulation were seen in Group 1 but not in Group 2. The complement-receptor-positive cells and antibody formations were unaffected in both groups.

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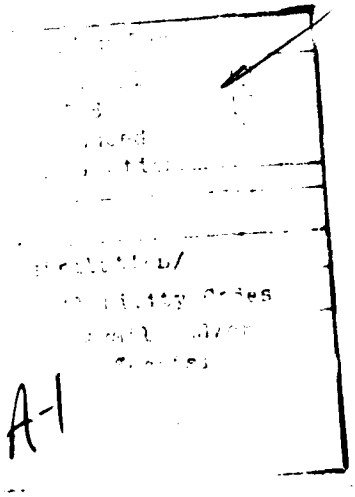

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EFFECTS OF LONG-TERM LOW-LEVEL RADIOFREQUENCY RADIATION EXPOSURE ON RATS

VOLUME 5. EVALUATION OF THE IMMUNE SYSTEM'S RESPONSE

INTRODUCTION

Human populations in consumer, military, and industrial environments are continually exposed to a rapidly increasing number of microwave-emitting devices. The technology has far outpaced the research into biohazardous implications. More than 6,000 articles have been published on the subject, but most have dealt with exposures of a few animals or of short duration and thus provide little insight into the long-term cumulative effects.

During the past three years, the Bioelectromagnetics Research Laboratory at the University of Washington has conducted the largest single evaluative study on the bioeffects of microwaves. The goal of this project was to investigate purported adverse effects on health after long-term exposure to pulsed-microwave radiation. The major emphasis was to expose a large population of experimental animals to microwave radiation throughout their lifetimes and monitor them for cumulative effects on general health and longevity. This report, fifth in a series covering the project, deals particularly with the investigation into the immune system's response.

An increasing number of studies have been published concerning the immunological responses of various experimental animals to microwave irradiation. These studies provide a basic framework for inquiry but, when viewed as a whole, reveal inconsistencies and inadequacies that stimulate controversy concerning the significance of basic findings with regard to potential hazard to human health.

Stimulatory effects indicated by the results of some studies have not been reproduced in others. Increased lymphoid blastogenesis followed mitogen stimulation in mice exposed to 25-kHz electric fields and in monkeys exposed to 27-MHz fields in studies by Bollinger et al. (1974) and

Prince et al. (1972) respectively. Increased numbers of peripheral lymphoblasts in exposed rats and mice were reported by both Czerski et al. (1974) and Huang et al. (1977). Studies by Liburdy (1979; 1980) indicated effects on lymphocytic circulation in mice from both radiofrequency radiation (RFR) and steroid treatment; he speculated that the thermal stress and thermoregulatory effects associated with steroid release were responsible for the RFR effects on the immune system. The studies of Smialowicz (1979) and Roszkowski et al. (1980) indicated that the changes in hemopoiesis and lymphopoiesis induced by microwave hyperthermia could be attributed mostly to nonspecific stress responses or to direct thermal effect on immunocompetent cells. In vitro studies conducted by Roszkowski et al. (1979) indicate that the metabolism of lymphocytes and reticulo-endothelial cells is stimulated by hyperthermia at 38-40^{o}C, dramatically inhibited at 43^{o}C, and irreversibly damaged at higher temperatures. The changes in lymphoid blastogenic reactivity to mitogens and synthesis of antibodies by lymphocytes observed in these in vitro studies seem to have been influenced by deposition of microwave energy. This deposition may have been the basis also of the stimulatory effect of microwaves on the maturation of B-lymphocytes, leading to increased abundance of complement-receptor-positive cells (CRPC) (Schlagel et al. 1980), enhanced responsiveness to mitogens, and augmented plaque-forming ability (antibody production) (Wiktor-Jedrzejczak et al. 1980; Sulek et al. 1980; Liddle et al. 1980).

The above studies reported stimulatory effects that have not been reproduced in others. They also had apparent discrepancies among themselves, especially those performed at low field intensities, due in part to the variations in level and duration of exposure (Smialowicz 1979; Huang 1980; McRee et al. 1980; Hellstrom et al. 1981).

The stage of maturity of an animal's immune system may be a factor in determining susceptibility to modification by microwave irradiation. Smialowicz et al. (1982b) found no consistent differences in plaque-forming ability, mitogen response, or activity of natural-killer cells between irradiated and sham-irradiated groups exposed in utero to 2450-MHz 28-mW/cm^2 microwaves for 100 min daily from day 6 to day 18 of pregnancy, when they made immunological assessments in the mice at 3 and 6 weeks of age. In contrast, Wiktor-Jedrzejczak et al. (1977) had reported

alterations in mice following postnatal exposure: a mild stimulatory effect on splenic lymphocytes indicated by increases in responsiveness to mitogens and number of antibody-forming spleen cells. In rats, however, enhancement of lymphocytic responsiveness was found in animals exposed in utero at 425 MHz (Smialowicz et al. 1982a) as well as in those irradiated both in utero and during early postnatal life (Smialowicz 1979, Smialowicz et al. 1982b). The above studies indicate that postnatal irradiation increases lymphocytic responsiveness in mice, whereas in rats this effect occurs with either in utero or postnatal exposure. This variation between mice and rats may be related to species differences in maturation of the immune system.

Smialowicz et al. (1982b) suggest a tendency for modification of immunological competency tests in rats exposed to microwave radiation resulting in an SAR in excess of their basal metabolic rate. Bowhill (1981) affirms that exposure of animals to acute microwave irradiation or exposure lasting from one to several days results in stimulation of immune system activity; further, he speculates that such exposure elicits a biphasic reaction in the immune system. His supposition is not documented, however, by experimental data and is contrary to the findings in long-term exposure experiments conducted with mice (Bollinger et al. 1974), monkeys (Prince et al. 1972), and rats (Kunz et al. 1982).

The evaluative study we report here was of long-term low-level exposure of rats and consisted of a number of basic tests to detect profound immunological effects, as follows: (1) Evaluation of splenic lymphocyte populations with respect to numbers of B- and T-cell antigen-positive lymphocytes, and of complement receptor-bearing lymphocytes; (2) Evaluation of the response of spleen lymphocytes to the following mitogens: phytohemaglutinin (PHA), concanavalin A (ConA), pokeweed (PWM), lipopolysaccharide (LPS), and purified protein derivative of tuberculin (PPD); (3) Evaluation, by means of the direct plaque-forming-cell assay, of ability of splenic cells from experimental animals, after immunization, to produce antibodies against the sheep red blood cells (SRBC), a T-cell-dependent antigen.

EXPOSURE FACILITY

As part of the overall project, a unique exposure facility was prepared that allowed 200 rats to be maintained under specific-pathogen-free (SPF) conditions while housed in individual, circularly polarized waveguides. The exposure facility consisted of two rooms, each containing 50 waveguides for active exposure and 50 waveguides for sham exposure. Each room contained two 2450-MHz pulsed-microwave generators, each capable of delivering a maximum of 10 W average power at 800 pps with a 10-μsec pulse width. This carrier was square-wave modulated at an 8-Hz rate. The power-distribution system delivered 0.144 W to each exposure waveguide for an average power density of 480 $\mu W/cm^2$. Whole-body calorimetry, thermographic analysis, and power-meter-output analysis indicated that these exposure conditions resulted in average specific absorption rates (SARs) ranging from approximately 0.4 W/kg for a 200-g rat to 0.15 W/kg for an 800-g rat.

EXPERIMENTAL DESIGN

Two hundred male rats, obtained at 3 weeks of age from a commercial barrier-reared colony, were randomly assigned to exposed and sham-exposed treatment conditions. Exposure began at 8 weeks of age and continued for 25 months. Throughout this period, all surviving animals were bled at regular intervals and blood samples were analyzed for serum chemistry, hematological values, protein electrophoresis patterns, and thyroxine (T_4) and serum corticosterone levels. Body weight, food and water consumption, oxygen consumption, and carbon dioxide production were measured daily on subpopulations of the exposed and sham-exposed animals. Activity was assessed in an open-field apparatus at regular intervals throughout the study. After 13 months 10 rats from each treatment condition were killed for immunological competence testing, whole-body analysis, and gross and histopathological examinations. At the end of 25 months the surviving 10 rats from each group were killed and similar analyses were made.

INTERIM EVALUATION OF THE IMMUNE SYSTEM

As part of the RFR bioeffects program, the following immunological tests were performed coincident with the interim killing of 10 animals from each treatment group: response of splenic lymphocytes to various mitogens, plaque-forming ability, complement-receptor formation, and enumeration of B- and T-cells. These are described in the following sections, together with the results.

Mitogen-Stimulation Assay

As an in vitro measure of cell-mediated immunity, DNA synthesis, and cell division, splenic lymphocytes were stimulated with various B- and T-cell-specific mitogens. Two T-cell-specific mitogens were used, the plant lectins ConA and PHA, and two B-cell-specific mitogens: bacterial LPS of E. coli and PPD of tuberculin. In addition, PWM was used, a plant lectin that is primarily B-cell-specific but that also stimulates some T-cells. The techniques used were modifications of methods described by Bloom and David (1976) and Janassy and Greaves (1971).

Materials

Media:	Extra-high amino acid (EHAA) containing -	
	4×10^{-5} M 2 Me	
	7.5 MM HEPES(N-2-hyroxyethylpiperazine-N[1] 2-ethanesulfonic acid)	
	$NaHCO_3$ to pH 7.3	
^{3}H-thymidine:	stock 5 μCi/mmol - dilution of 1:10	
Mitogen preparations:	PHA	0.2 mg/ml
	ConA	2.0 μg/ml
	LPS	0.1 mg/ml
	PPD	0.05 mg/ml
	PWM	1:2000 dilution of stock GIBCO

Procedure

During the course of the interim kill, 20 animals (10 exposed and 10 sham exposed) were removed from the waveguides at approximately 1.5-min intervals, taken to the adjoining necropsy room, anesthetized with halothane and nitrous oxide, and exsanguinated via the brachial artery. The spleens were removed, and an approximate 8% section was taken for histopathological analysis. The remaining portion of each spleen was packed in ice and delivered to the laboratory for analysis within 15 to 30 min of spleen removal.

The spleens were minced, forced through a fine-mesh wire screen, and suspended to 15 ml; the suspension was passed over gauze to remove large debris. Each spleen cell (SPC) suspension was then centrifuged at 1000 rpm for 10 min. The pellets were exposed to 5 ml of 0.83% NH_4Cl + 0.017 M Tris solution for 5 min at room temperature for lysis of red blood cells. The cells were then resuspended to 15 ml and the suspension was centrifuged, the cells were resuspended and the suspension was centrifuged, and finally the cells were resuspended and counted.

Each SPC preparation was then diluted to a concentration equal to 1.5×10^6/ml. The various mitogens had previously been prepared to the concentrations indicated in the Materials section. To the wells of a Falcon #3040 plate, 0.1 ml of SPC and 0.1 ml of a specific mitogen were added. The peak response for PHA, ConA, LPS, and PWM had been previously determined to be 3 days, so the cells prepared with these mitogens were incubated at 37°C for 3 days. The peak response for PPD had been found to be 6 days, so these cultures were incubated for 6 days. In all instances, 10 µl of ^{3}H-thymidine working solution was added to each culture 18 h prior to harvest, and incubation was allowed to continue for the remaining hours.

At the end of the incubation period, the labeled cells from each culture were collected and put on filter paper. The unincorporated ^{3}H-thymidine was washed away during harvesting by a Multiple Automated Sample Harvester (MASH II, Microbiological Associates). The filter paper discs were dried and placed in Liquifluor scintillation fluid and counted for 10 min/tube in a Packard beta counter. All mitogen-treatment determinations on each SPC were made in triplicate and averaged.

The stimulation index was then computed for each culture as the mean counts/minute (cpm) for the mitogen-treatment condition divided by the mean cpm for the media-only treatment condition:

$$S.I. = \frac{\text{cpm for mitogen-treated group}}{\text{cpm for media-treated groups}}$$

The data will be presented and analyzed on the basis of the stimulation index and not the raw count data. Owing to illness and failure of technique, one animal from each group provided data that significantly differed from those representative of either group; data from these animals were excluded from further analysis.

Results

The following results of the various mitogen-stimulation assays are presented in Table 1: the mean of the stimulation index associated with each mitogen, standard deviation, standard error of the mean, size of the sample, and maximum and minimum observed values; also, the *t*-statistics for the between-group comparisons. The comparisons are graphically depicted in Fig. 1.

With significance evaluated at the .05 level, the exposed animals showed a nonsignificant increase in stimulation index for PHA; significant increases for LPS, ConA, and PWM; and a significant decrease for PPD. However, a multivariate analysis of all five mitogen treatments yielded a nonsignificant result, examined by Hotelling's T^2 statistic ($F = 1.93$, $p = .162$, d.f. = 5, 12).

A problem associated with the preceding analysis is the high degree of variability within groups. The standard deviations are often noncomparable between groups and appear proportional to the means. In addition, the data are highly skewed. In an attempt to alleviate these problems, we transformed the data using the log transformation and conducted further analysis using the log values of the stimulation indices. The transformed data are shown in Table 2 and graphically presented in Fig. 2.

TABLE 1. RAW STIMULATION INDICES FOR FIVE MITOGENS

Mitogen		Exposed	Sham	Statistic[a]	p	d.f.
PHA	Mean	5.66	4.52	Mean comparison		
	S.D.	0.942	1.37	t = 2.04	.058	16
	S.E.M.	0.314	0.458			
	Sample Size	9	9			
	Maximum	6.7	6.0			
	Minimum	3.7	2.0			
LPS	Mean	6.06	3.67	Mean comparison		
	S.D.	2.89	.951	t = 2.35	.032	16
	S.E.M.	0.964	.317			
	Sample Size	9	9			
	Maximum	11.5	5.5			
	Minimum	3.3	2.1			
ConA	Mean	17.0	10.7	Mean comparison		
	S.D.	6.14	3.67	t = 2.65	.018	16
	S.E.M.	2.05	1.22			
	Sample Size	9	9			
	Maximum	30.5	19.3			
	Minimum	10.2	6.9			
PWM	Mean	6.41	4.61	Mean comparison		
	S.D.	1.94	1.08	t = 2.43	.027	16
	S.E.M.	0.658	0.360			
	Sample Size	9	9			
	Maximum	9.2	6.2			
	Minimum	3.6	3.0			
PPD	Mean	2.74	6.98	Mean comparison		
	S.D.	0.905	4.71	t = -2.65	.018	15
	S.E.M.	0.302	1.57			
	Sample Size	9	8			
	Maximum	3.6	13.7			
	Minimum	1.4	2.0			
HOTELLING's T SQUARE =				F = 1.93	.162	5,11

[a]Mean differences between groups were evalulated with the Student's t-test.

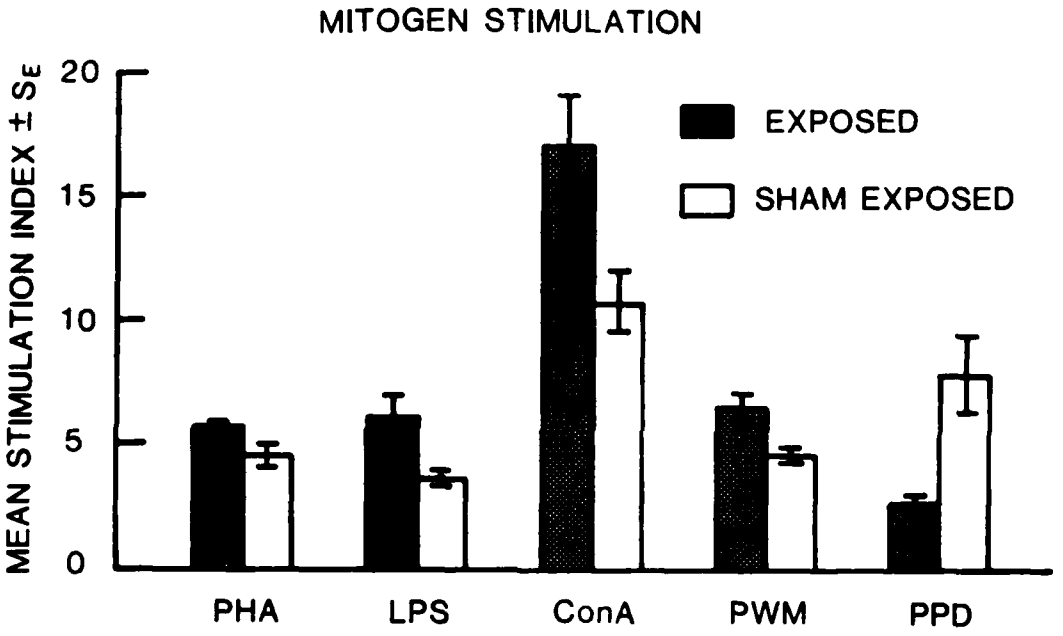

Fig. 1. Mean stimulation indices for exposed and sham-exposed animals for treatments with five mitogens.

TABLE 2. LOG-TRANSFORMED STIMULATION INDICES FOR FIVE MITOGENS

Mitogen		Exposed	Sham	Statistic[a]	p	d.f.
LPHA	Mean	1.72	1.46	Mean comparison		
	S.D.	0.185	0.369	t = 1.91	.075	16
	S.E.M.	0.062	0.123			
	Sample Size	9	9			
	Maximum	1.91	1.79			
	Minimum	1.31	0.693			
LLPS	Mean	1.71	1.27	Mean comparison		
	S.D.	0.442	0.271	t = 2.56	.021	16
	S.E.M.	0.147	0.090			
	Sample Size	9	9			
	Maximum	2.44	1.70			
	Minimum	1.19	.74			
LConA	Mean	2.78	2.32	Mean comparison		
	S.D.	0.331	0.299	t = 3.06	.008	16
	S.E.M.	0.110	0.099			
	Sample Size	9	9			
	Maximum	3.42	2.96			
	Minimum	2.32	1.93			
LPWM	Mean	1.81	1.50	Mean comparison		
	S.D.	0.312	0.240	t = 2.38	.030	16
	S.E.M.	0.104	0.080			
	Sample Size	9	9			
	Maximum	2.33	1.82			
	Minimum	1.28	1.10			
LPPD	Mean	0.949	1.90	Mean comparison		
	S.D.	0.389	0.654	t = -3.70	.002	15
	S.E.M.	0.130	0.231			
	Sample Size	9	8			
	Maximum	1.28	2.62			
	Minimum	0.337	0.693			
HOTELLING's T SQUARE = 32.12				F = 4.52	.017	5,11

[a]Mean differences between groups were evaluated with the Student's t-test.

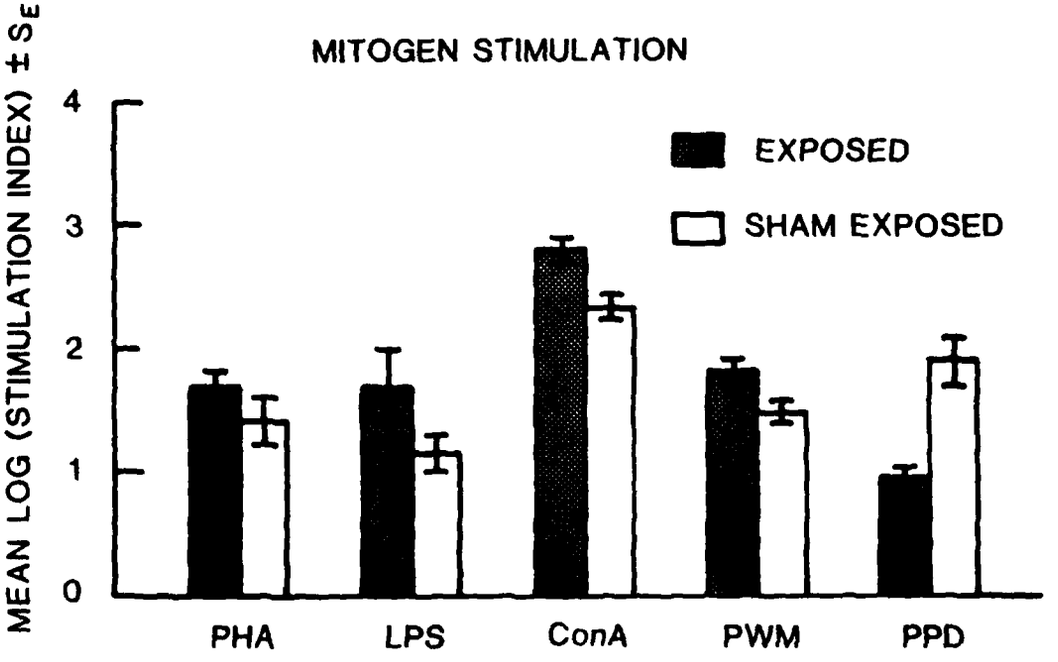

Fig. 2. Mean log stimulation indices for exposed and sham-exposed animals for treatments with five mitogens.

For the exposed animals, at the $p<.05$ level the log values of the stimulation indices showed a nonsignificant increase for PHA and significant increases for LPS and PWM; at the $p<.01$ level the increase for ConA and the decrease for PPD were both significant. The multivariate analysis of all five measures was significant on the basis of Hotelling's T^2 statistic ($F = 4.52$, $p = .017$, d.f. = 5, 11). From the pairwise comparisons it is apparent that ConA and PPD contributed substantially to the overall differentiation of the two groups.

Inspection of the data on mitogen stimulation showed that the correlation matrices appear to differ between the exposed and sham-exposed groups. The correlation matrices for the raw data and transformed data are presented in Tables 3 and 4 respectively. For the exposed group, PHA was negatively correlated with LPS, ConA, and PWM, but positively correlated with PPD. For the sham-exposed group, however, PHA was positively correlated with LPS, ConA, and PWM, and negatively correlated with PPD. These differences were not assessed for statistical significance, as no satisfactory test exists for use with such small samples. The effect on Hotelling's T^2, which assumes equal correlation matrices, is not substantial when sample sizes are nearly equal (Ito and Schull 1964).

TABLE 3. CORRELATION MATRICES FOR RAW STIMULATION INDICES FOR FIVE MITOGENS FOR EXPOSED AND SHAM-EXPOSED GROUPS

	Mitogen	PHA	LPS	ConA	PWM	PPD
	PHA	1.0000				
	LPS	-0.6095	1.0000			
EXPOSED	ConA	-0.5342	0.9094	1.0000		
	PWM	-0.3456	0.8940	0.8882	1.0000	
	PPD	0.7277	-0.4501	-0.2433	-0.1416	1.0000
	PHA	1.0000				
	LPS	0.8458	1.0000			
SHAM	ConA	0.6872	0.4466	1.0000		
	PWM	0.7899	0.7285	0.7602	1.0000	
	PPD	-0.5934	-0.4135	-0.6074	-0.1814	1.0000

TABLE 4. CORRELATION MATRICES FOR LOG TRANSFORMATIONS OF STIMULATION INDICES FOR FIVE MITOGENS FOR EXPOSED AND SHAM-EXPOSED GROUPS

	Mitogen	PHA	LPS	ConA	PWM	PPD
	PHA	1.0000				
	LPS	-0.5798	1.0000			
EXPOSED	ConA	-0.4755	0.8462	1.0000		
	PWM	-0.3195	0.8304	0.9100	1.0000	
	PPD	0.6848	-0.4431	-0.1147	-0.0140	1.0000
	PHA	1.0000				
	LPS	0.9028	1.0000			
SHAM	ConA	0.7208	0.5958	1.0000		
	PWM	0.7913	0.7543	0.8087	1.0000	
	PPD	-0.3866	-0.4514	-0.1813	0.1322	1.0000

Enumeration of B- and T-Cells

Splenic cells obtained from the 10 exposed and the 10 sham-exposed animals in the interim kill were prepared, and the B- and T-cell populations were enumerated by modifications of the methods described by Bloom and David (1976), Ainti et al. (1975), Basten et al. (1972), and Balch and Feldman (1974).

Materials

Antibodies: Goat antirat IgG fluorescein isothiocanate (FITC) - labelled $F(ab)^2$ fraction
Rabbit antimouse brain
Goat antirabbit IgG FITC-labelled

Procedure

Splenic cell suspensions were prepared for each animal as previously described for the mitogen-stimulation assays. Then 2×10^6 SPC were pelleted in 15-ml centrifuge tubes and stained for detection of either B- or T-cells by direct- and indirect-immunofluorescent techniques respectively.

B-Cell Preparation: The primary antibody was the $F(ab)^2$ fraction of the goat antirat IgG. For staining, 50 µl of the labeled antibody was added to one of the SPC pellets, and the suspension was incubated on ice for 30 min. The cells were then washed by suspension in 15 ml of medium, the suspension was centrifuged at 1000 rpm for 10 min, and the cells were resuspended in 200 ml of medium containing 0.1% sodium azide. An equal volume of 2% paraformaldehyde was added to the SPC. Counting proceeded under a fluorescent microscope.

T-Cell Preparation: T-cells were detected by the indirect-immunofluorescence technique. The SPC pellet was suspended in 500 µl of rabbit antimouse brain antibody, and the suspension was incubated on ice for 30 min. The cells were then suspended in 15 ml of medium, and the suspension was centrifuged for 10 min at 1,000 rpm. The pellet was resuspended in 50 µl of FITC-labelled goat antirabbit IgG and incubated on ice for 30 min. Incubation was followed by suspension in 15 ml of medium,

centrifugation, and resuspension in 200 µl of medium (containing 0.1% sodium azide). An equal volume of 2% paraformaldehyde was added to the SPC, which were then counted under a fluorescent microscope. The percentages of B- and T-cells in the SPC population for each animal were calculated, and then the mean percentages were compared between irradiated and sham-irradiated animals.

Results

Figure 3 presents the mean percentages of B- and T-cells in the SPC population for each group. The exposed group had a significant enhancement of both B- and T-cell enumerations relative to the sham-exposed group. Table 5 presents the means, standard deviations, standard errors of the mean, sample sizes, and maximum and minimum values for both comparisons. The Student's t-test showed a significant mean difference for the B-cell population (t = 3.76, p = .002, d.f. = 16) and for the T-cell population (t = 3.48, p = .003, d.f. = 16). The results of the combined B-/T-cell enumeration test were also significant, judged by the Hotelling T^2 statistic (F = 8.07, p = .004, d.f. = 2, 15).

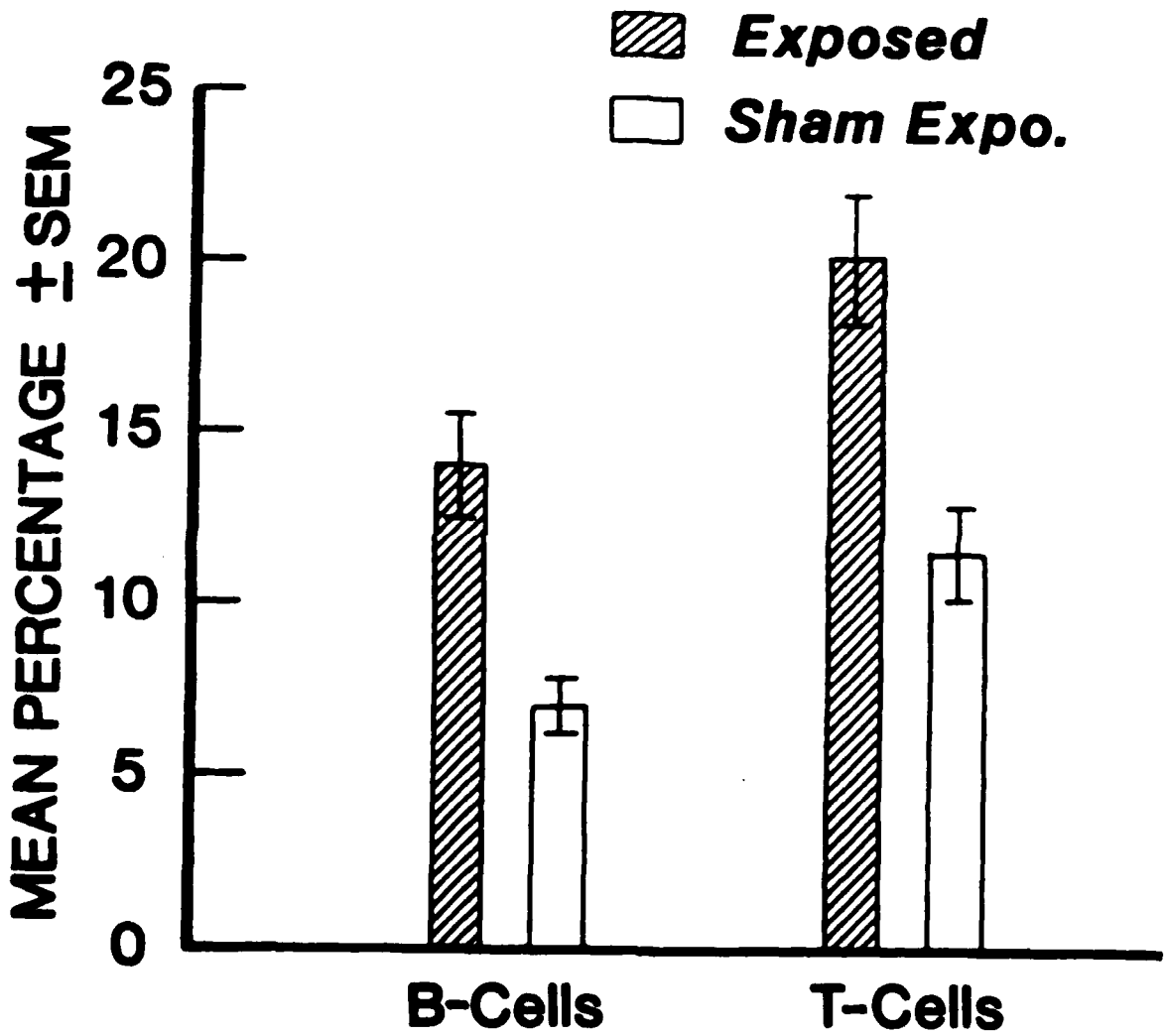

Fig. 3. Mean percentages of B-cells and T-cells within culture population of splenic lymphocytes for exposed and sham-exposed groups.

TABLE 5. B- AND T-CELL ENUMERATIONS (PERCENTAGES)

Population		Exposed	Sham	Statistic[a]	p	d.f.
B-CELL	Mean	14.39	7.33	Mean comparison		
	S.D.	5.02	2.56	t = 3.76	.002	16
	S.E.M.	1.67	.853			
	Sample Size	9	9			
	Maximum	22.1	11.7			
	Minimum	6.7	3.5			
T-CELL	Mean	19.18	11.40	Mean comparison		
	S.D.	5.24	4.19	t = 3.48	.003	16
	S.E.M.	1.75	1.40			
	Sample Size	9	9			
	Maximum	27.0	20.0			
	Minimum	9.2	5.3			
HOTELLING's T-SQUARE = 17.21				F = 8.07	.004	2,15

[a]Mean differences between groups were evaluated with the Student's t-test.

Complement-Receptor Assay

Splenic cells obtained from the 10 exposed and the 10 sham-exposed animals in the interim kill were assayed for the ability to form CRPC. The techniques used were modifications of the methods described by Bianco, Patrick, and Mussenzweig (1970).

Materials

Media:	RPMI pH 7.2
	RPMI pH 7.2 + 0.01 M EDTA
Complement:	Fresh A/Sn serum
Antibody:	Anti-SRBC antibody

Procedure

Splenic cells were prepared as described for the mitogen-stimulation assay and diluted to a concentration of 1×10^7/ml. SRBC in Alsever's solution were washed three times by suspension in medium, the suspension was centrifuged at 1800 rpm, and the cells were resuspended. Then 120 µl of packed SRBC was suspended in 10 ml of medium and an additional 10 ml of anti-SRBC antibody (stock diluted 1:5) was added. The SRBC were incubated at 37°C for 40 min, then washed three times as described above. After the final wash, the SRBC-antibody cells (EA) were suspended in 6 ml of RPMI + 0.01 M EDTA. As the source of complement, 6 ml of undiluted, freshly bled A/Sn serum was added to the EA suspension. This suspension (EAC) was incubated at 37°C for 40 min, then washed three times and resuspended in 6 ml of RPMI containing 0.01 M EDTA.

For the complement-receptor assay, 0.2 ml of SPC (1×10^7/ml) was mixed with 0.2 ml of EAC and incubated at 37°C for 1 h. The SPC-EAC suspension was stained with 0.1% toluidine blue, and the number of rosettes were counted in a hemocytometer. The percentage of CRPC was calculated as:

$$\% \text{ CRPC} = \frac{\text{total number of rosettes}}{\text{total number of SPC}}$$

The total number of CRPC/spleen was calculated as:

$$\text{total CRPC} = \%\ \text{CRPC} \times \frac{\text{total SPC}}{100}$$

Results

The percentages of CRPC and total numbers of CRPC/spleen for exposed and sham-exposed groups are presented in Fig. 4. The decreases in both percentage of CRPC and CRPC/spleen among the exposed animals were nonsignificant as analyzed by the Student's *t*-test.

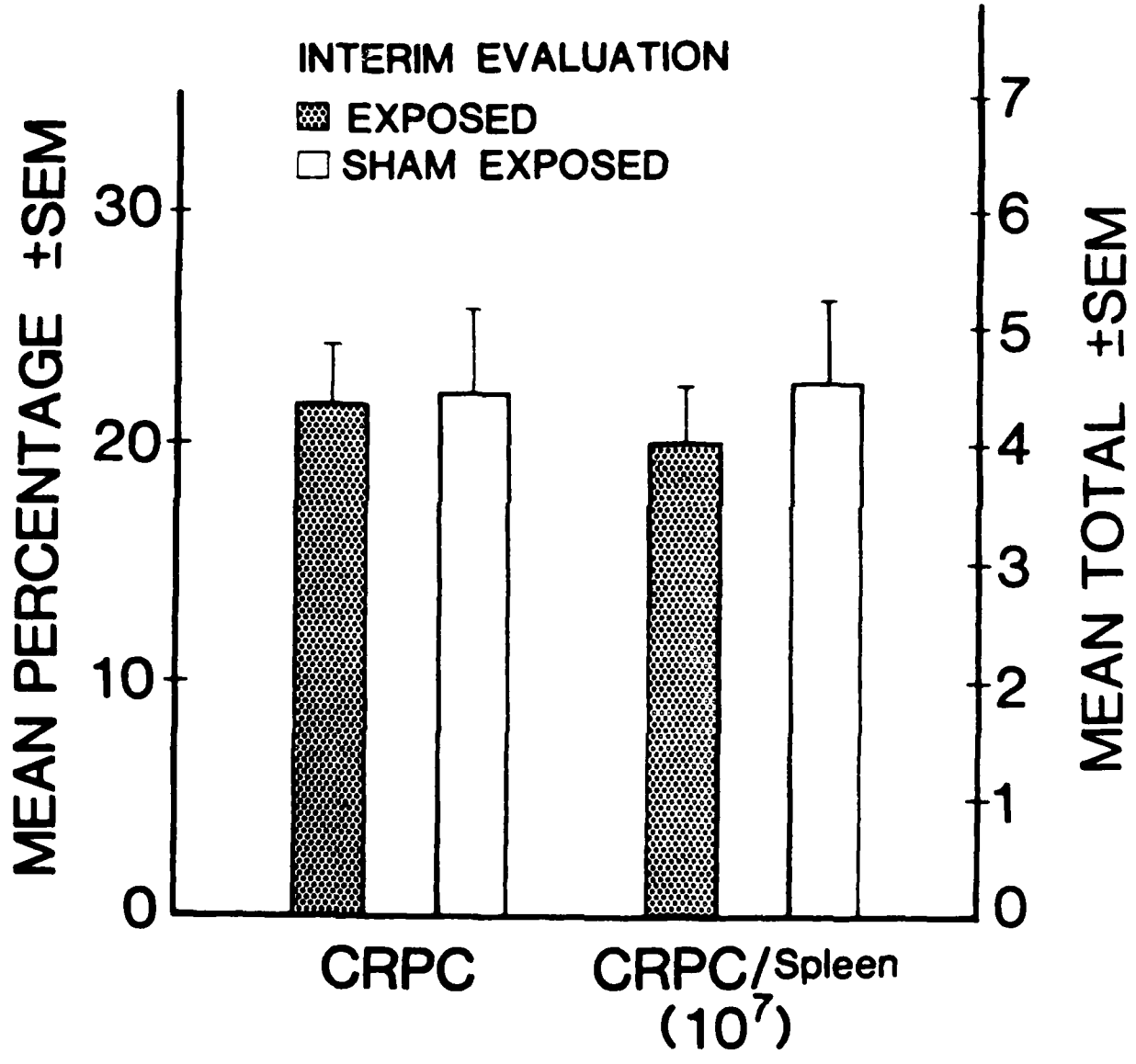

Fig. 4. Mean percentages of CRPC for exposed and sham-exposed groups.

Plaque Assay

Splenic cells obtained from the 10 exposed and the 10 sham-exposed animals in the interim kill were assayed in duplicate for the ability to form plaque-forming cells in response to SRBC. The techniques used were modifications of the methods described by Jerne et al. (1963) and Dresser and Wortis (1965).

Materials

Antigen:	SRBC
Media:	RPMI
Plates:	Lab-tek tissue culture chamber slides
Complement:	Guinea pig serum (GPC)

Procedure

Half of the 10 exposed and 10 sham-exposed animals had been previously selected and intraperitoneally injected with 2 ml of 10% SRBC solution 6 days before being killed. The other half were injected intraperitoneally with 2 ml of phosphate-buffered saline (PBS). After the kill, splenic cells were prepared as described for the mitogen-stimulation assay and diluted to 2×10^7/ml. The SRBC were washed as described for the complement-receptor assay, and a 10% solution was prepared. A 1% agarose solution in RPMI was prepared and kept in a constant-temperature ($40^{\circ}C$) water bath. To prewarmed ($40^{\circ}C$) tubes were added 200 µl of agar, 40 µl of 10% SRBC, and 50 µl of SPC. Of this mixture 100 µl was added to duplicate cultures in the chambers of a slide.

When the agar solidified, 35 µl of GPC (diluted 1:5) was added to each chamber. The chambers were then incubated at $37^{\circ}C$ for 1.5 h, at which time plaques were counted using a dissecting microscope. The total plaques per spleen were calculated as follows:

$$\text{plaques/spleen} = \frac{\text{total SPC} \times \text{average plaques/chamber}}{\text{SPC/chamber}}$$

Results

The animals immunized with PBS produced no plaques, whereas the exposed animals immunized with SRBC produced an increased number of plaques per spleen, with a mean of 8.14 $\pm$ 1.8. The mean for the sham-exposed animals was 5.7 $\pm$ 1.4. This apparent increase was nonsignificant (t = 1.0, p > .30, d.f. = 8), examined by the Student's t-test.

Discussion

The results of the mitogen-stimulation studies following 13 months of exposure reveal a significant difference between groups in the patterns of response to various B- and T-cell specific mitogens. The exposed animals had a nonsignificant increase in response to PHA and a significant increase (p = .05) in response to LPS and PWM. They had a significantly increased response to ConA and a decreased response to PPD (p = .01). These results suggest a selective effect of RFR on the lymphoreticular system.

Compared with the sham-exposed group, the exposed experimental animals had a significant increase in both splenic B- and T-cells. This increase suggests a general stimulation of the lymphoid system in the animals exposed to RFR.

No significant differences were seen between the exposed and sham-exposed rats in percentage of CRPC in the spleen. In the animals immunized with SRBC, a slight but statistically insignificant increase in plaques per spleen was seen for the exposed animals relative to the sham exposed. This result indicates no grossly significant impairment of the reticuloendothelial system, which first processes the SRBC antigen, and no deficiency in the B-cells' ability to produce antibodies in the presence of T-cells, as this antigen is T-cell dependent.

In summary, the screening studies of the immune system in rats after 13-month exposure to low-level RFR suggested a general increase in the abundance of splenic B- and T-cells, as well as a selective stimulation of their ability to respond to mitogens. There was no impairment of the

reticuloendothelial system nor inability to produce antibodies and form CRPC. The incidence of lymphoreticular disorders and neoplasia in relation to this apparent difference in response to selective mitogens as these animals age will be evaluated for possible significance.

TERMINAL EVALUATION OF THE IMMUNE SYSTEM

The same immunological tests conducted during the interim kill were performed coincident with the terminal kill of 10 animals from each treatment group after 25 months of exposure: evaluations of (1) response of splenic lymphocytes to various mitogens, plaque-forming ability, complement-receptor formation; and (2) B- and T-cell populations. These tests and their results are described in the following sections.

Mitogen-Stimulation Assay

Splenic lymphocytes were stimulated with the same B- and T-cell specific mitogens: the plant lectins ConA and PHA for T-cell populations; bacterial LPS of E. coli and PPD of tuberculin for B-cell populations; and PWM for both populations. The animals were killed and spleens collected according to the protocol used in the interim kill, with the modification that the entire spleen and the section removed for histopathology were weighed.

Evaluation of the mitogen test cultures and the stimulation indices indicated that all of the lymphocyte cultures failed to grow and there was no response to any of the mitogens. Because the RFR bioeffects program provided for only 10 exposed and 10 sham-exposed animals to be screened for immunological effects on a single day during the terminal kill, the mitogen-stimulation studies could not be repeated. No technical problems were encountered with the other immunological tests performed.

Enumeration of B- and T-Cells

Splenic cells obtained from the 10 exposed and the 10 sham-exposed animals were prepared, and the B- and T-cell populations were enumerated using the procedures described for the interim-kill evaluations.

Table 6 presents the percentages and total numbers of B- and T-cells in the SPC populations of the exposed and sham-exposed animals. Table 7 presents the means, standard deviations, standard errors, and maximum and minimum values for the exposed and sham-exposed groups. The Student's *t*-test showed the mean differences in percentages and absolute numbers for the B-cell population to be nonsignificant. The B- and T-cell enumeration tests were found nonsignificant, judged by the Hotelling T^2 statistic.

TABLE 6. PERCENTAGES AND TOTAL NUMBERS OF B- AND T-CELLS IN SPLEENS

	Rat	% B-cells per spleen	Total B-cells x 10^7 per spleen	% T-cells per spleen	Total T-cells x 10^7 per spleen
	1	31.7	5.4	19.4	3.2
	2	44	7.4	21.5	3.6
	3	23	6.4	4.9	1.3
	4	40	7.2	11.5	2.0
SHAM	5	53	11.6	12.2	2.6
	6	33	6.9	12.2	2.6
	7	46.9	11.2	6.2	1.4
	8	39.2	3.5	7.0	0.6
	9	39.5	9.8	4.6	1.1
	10	45.2	7.6	5.4	0.9
	11	39	5.4	10.5	1.4
	12	43.6	18.2	7.9	1.2
	13	38.2	6.8	10.1	1.8
	14	53.1	11.6	1.6	0.35
EXPOSED	15	34.6	7.2	8.0	1.6
	16	40	11.5	14.4	4.1
	17	33.6	5.3	8.4	1.3
	18	33.3	5.6	6.8	1.1
	19	38.2	6.8	10.6	1.8
	20	39	7.0	10.9	1.9

TABLE 7. PERCENTAGES AND ABSOLUTE COUNTS OF B- AND T-CELLS FOR EXPOSED AND SHAM-EXPOSED GROUPS

Population		Exposed	Sham	Statistic[a]	p	d.f.
B-CELL	Mean	39.26	39.57	Mean comparison		
PERCENT	S.D.	6.10	8.58	t = -0.09	.93	18
	S.E.M.	1.93	2.71			
	Sample Size	10	10			
	Maximum	53.1	53.0			
	Minimum	33.3	23.0			
T-CELL	Mean	8.92	10.59	Mean comparison		
PERCENT	S.D.	3.35	6.26	t = -0.74	.46	18
	S.E.M.	1.06	1.98			
	Sample Size	10	10			
	Maximum	14.4	22.5			
	Minimum	1.60	4.60			
B-CELL	Mean	9.28	8.25	Mean comparison		
TOTAL	S.D.	4.37	2.74	t = 0.63	.54	18
	S.E.M.	1.38	0.87			
	Sample Size	10	10			
	Maximum	19.59	12.80			
	Minimum	5.75	3.78			
T-CELL	Mean	1.80	2.05	Mean comparison		
TOTAL	S.D.	1.02	1.07	t = -0.53	.60	18
	S.E.M.	0.32	0.34			
	Sample Size	10	10			
	Maximum	4.43	3.72			
	Minimum	0.44	0.65			
HOTELLING's T SQUARE = 1.15				F = 0.24	.91	4,15

[a]Results of individual Student's t-test evaluations and of an overall comparison based on the Hotelling T^2 statistic

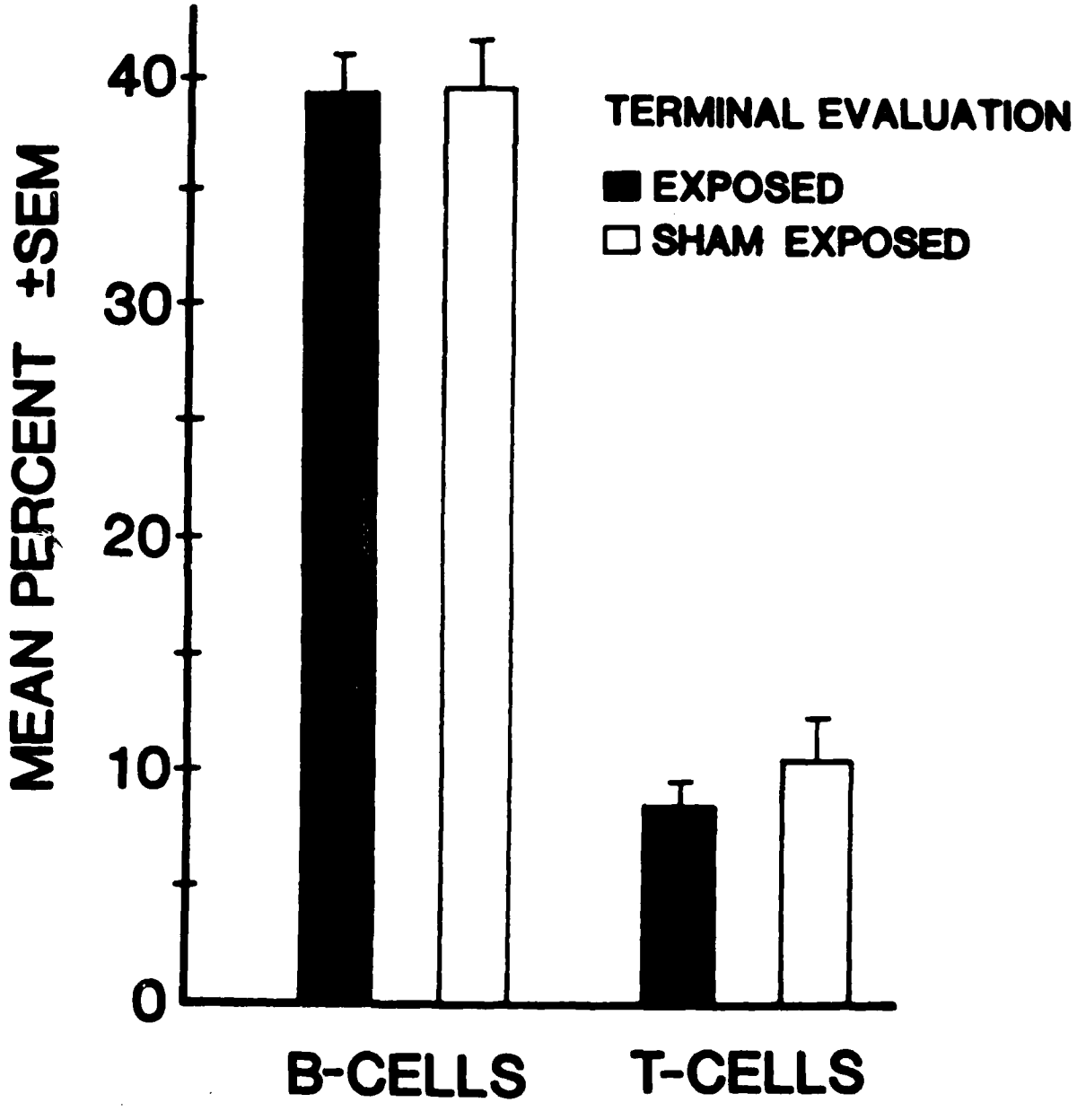

Fig. 5. Mean percentages of B-cells and T-cells within the population of splenic lymphocytes for exposed and sham-exposed groups.

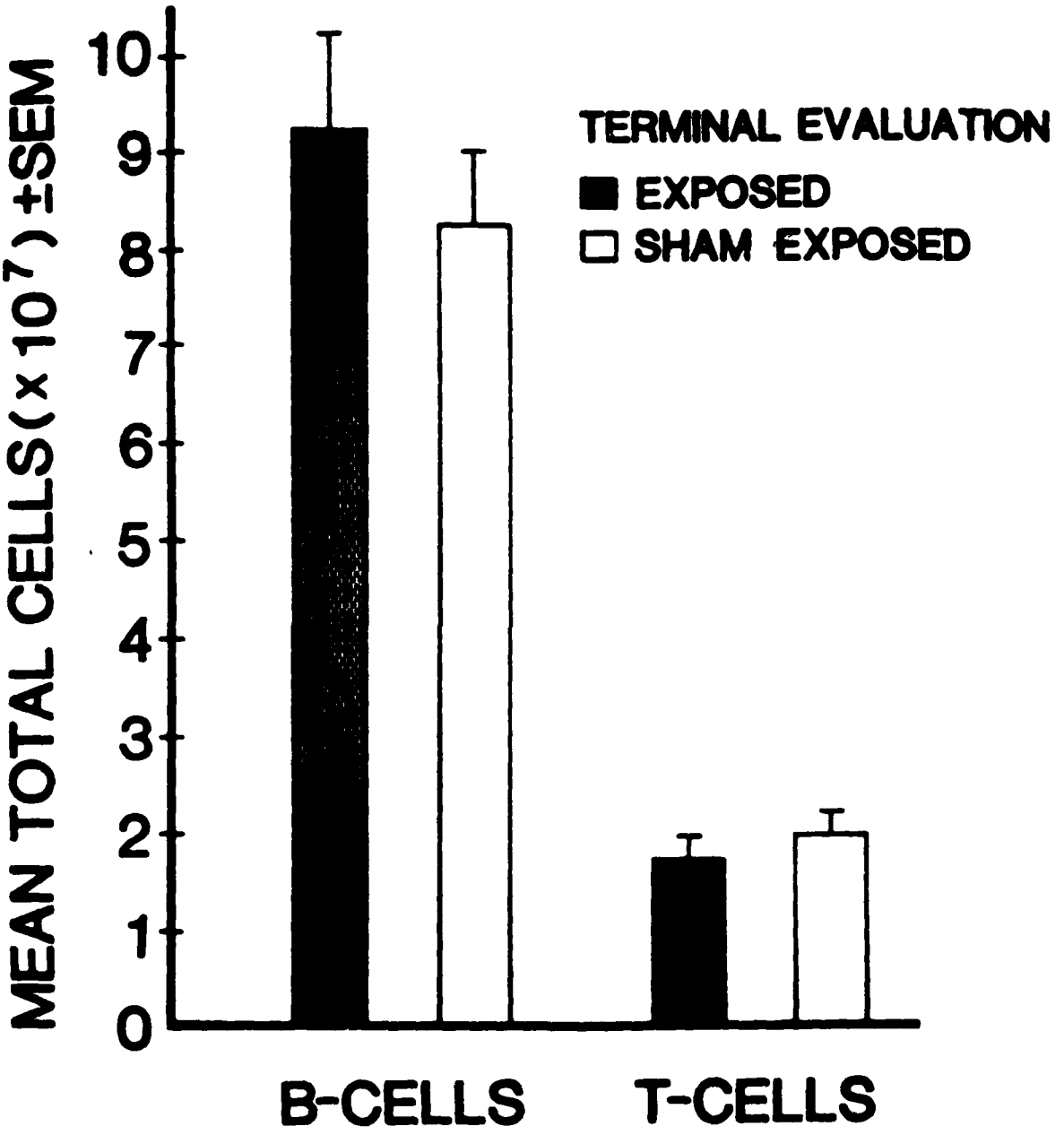

Fig. 6. Mean total numbers of B-cells and T-cells within the population of splenic lymphocytes for exposed and sham-exposed groups.

Complement-Receptor Assay

Spleen cells obtained from the 10 exposed and 10 sham-exposed animals in the terminal kill were assayed, using the procedures described for the interim-kill evaluation, for the ability to form CRPC.

The percentages of CRPC and total numbers of CRPC/spleen are presented in Table 8. The mean percentages and total numbers of CRPC per spleen are compared for the exposed and sham-exposed groups in Fig. 7. The decreases in both percentage and total number of CRPC among the exposed animals were nonsignificant judged by the Student's t-test.

TABLE 8. PERCENTAGES AND ABSOLUTE NUMBERS OF CRPC IN SPLEENS

	Rat	% CRPC	Total CRPC/spleen 10^7
	1	10.7	1.7
	2	32.4	5.5
	3	44	12.3
	4	43	7.7
SHAM	5	39.4	8.6
	6	18.7	3.9
	7	49	11.9
	8	22.8	2.0
	9	46.8	11.7
	10	38.2	6.4
	11	43.9	6.1
	12	27.4	4.3
	13	35.9	6.4
	14	37.5	8.2
EXPOSED	15	39.1	8.2
	16	8.2	2.3
	17	34.2	5.4
	18	47.7	8.1
	19	20.0	3.6
	20	31	5.5

TABLE 9. PERCENTAGES AND ABSOLUTE COUNTS OF CRPC FOR EXPOSED AND SHAM-EXPOSED GROUPS

Population		Exposed	Sham	Statistic[a]	p	d.f.
CRPC PERCENT	Mean	32.29	34.50	Mean comparison		
	S.D.	11.53	12.99	t = -0.40	.69	18
	S.E.M.	3.65	4.11			
	Sample Size	10	10			
	Maximum	47.70	49.00			
	Minimum	8.20	10.70			
CRPC TOTAL	Mean	6.33	7.65	Mean comparison		
	S.D.	2.20	4.21	t = -0.88	.39	18
	S.E.M.	0.69	1.33			
	Sample Size	10	10			
	Maximum	9.01	13.13			
	Minimum	2.43	1.81			
HOTELLING's T SQUARE = 0.33				F = 0.16	.85	2,17

[a]Results of individual Student's t-test and of an overall comparison based on the Hotelling T^2 statistic

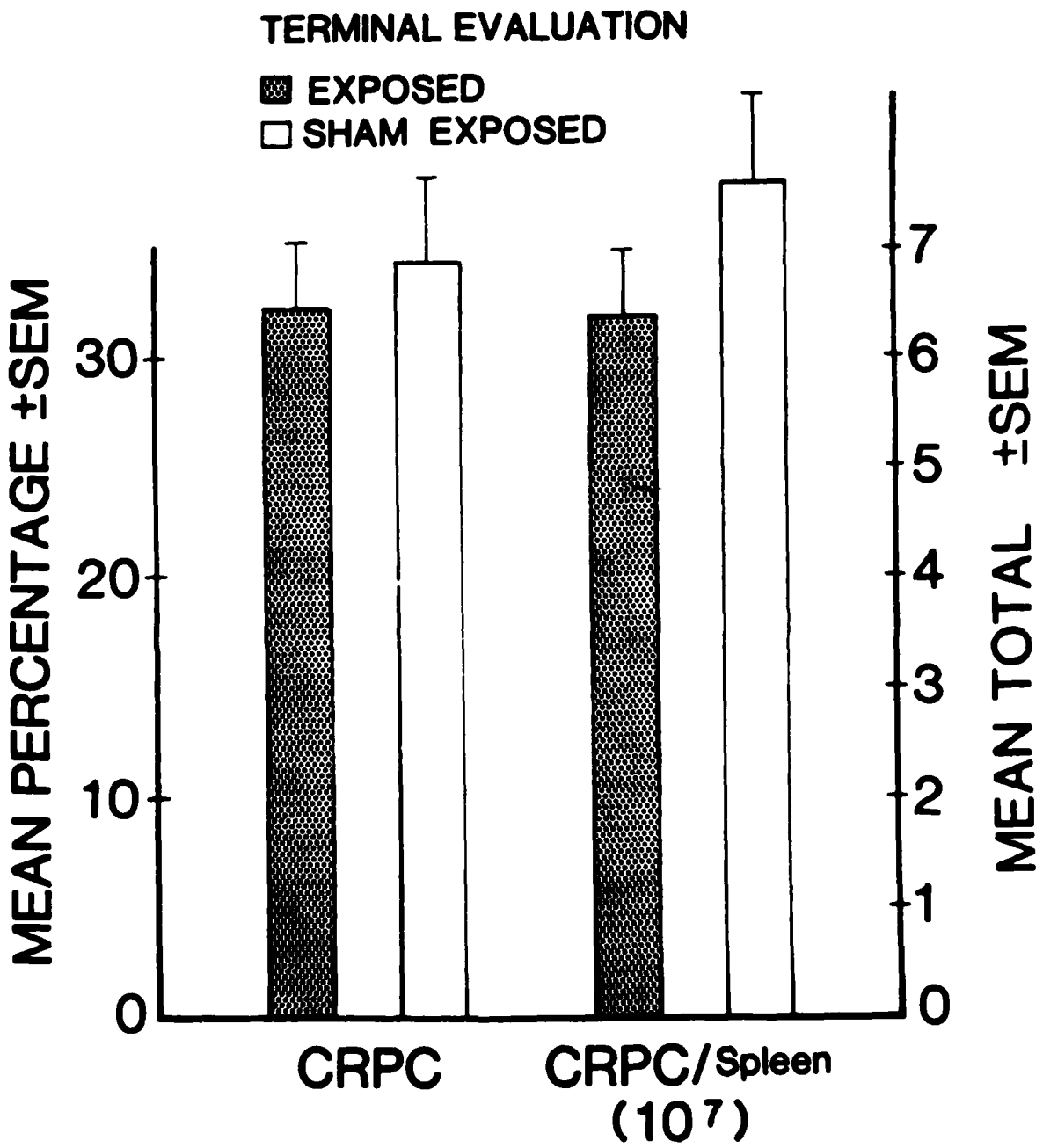

Fig. 7. Mean percentages and total numbers of CRPC in spleens of exposed and sham-exposed groups.

Plaque Assay

Splenic cells obtained from the 10 exposed and the 10 sham-exposed animals in the terminal kill were assayed in duplicate, using the procedures described for the interim-kill evaluation, for the ability to form plaque-forming cells in response to SRBC.

The total number of plaques/chamber and the computed total number of plaque-forming cells per spleen for each of the rats are presented in Table 10. The total splenic cell counts for the experimental animals are presented in Table 11. The animals immunized with PBS produced only artifactual counts of 0 to 3 plaquelike areas per chamber.

Analysis of data from the SRBC-injected animals indicated there were no significant differences between the exposed and sham-exposed treatment conditions based on plaques/chamber ($t = -1.175$, $\underline{p} = .279$, d.f. = 8) or plaques/spleen ($t = 1.755$, $\underline{p} = .117$, d.f. = 8).

TABLE 10. PERCENTAGES AND ABSOLUTE NUMBERS OF PLAQUE-FORMING CELLS IN SPLEENS

	Rat	Antigen	No. of plaques per chamber	Total plaques per spleen x 10^3
	1	SRBC	40	200
	2	SRBC	19	97
	3	SRBC	11.5	97
	4	SRBC	35	190
SHAM	5	SRBC	16	110
	6	PBS	2	12
	7	PBS	0	0
	8	PBS	3	8
	9	PBS	0.5	3.7
	10	PBS	0	0
	11	SRBC	24.5	100
	12	SRBC	10.5	57
	13	SRBC	31.5	170
	14	SRBC	3.5	20
EXPOSED	15	SRBC	5.5	35
	16	PBS	0	0
	17	PBS	0.5	2
	18	PBS	1	5
	19	PBS	0	0
	20	PBS	0.5	2

TABLE 11. TOTAL SPLENIC CELLS PER SPLEEN IN EXPOSED AND SHAM-EXPOSED RATS

	Rat	Total splenic cell counts (Total SPC x 10^8 per spleen
SHAM	1	1.7
	2	1.7
	3	2.8
	4	1.8
	5	2.2
	6	2.1
	7	2.4
	8	0.9
	9	2.5
	10	1.7
EXPOSED	11	1.4
	12	1.6
	13	1.8
	14	2.2
	15	2.1
	16	2.9
	17	1.6
	18	1.7
	19	1.8
	20	1.8

	Mean Lymphocyte Count
1-10 (sham)	$1.98 \times 10^8 \pm 0.17$
11-20 (exposed)	$1.89 \times 10^8 \pm 0.13$

Discussion

Data on response to mitogen stimulation were not available from the animals in the terminal kill because the lymphocyte cultures failed to grow and respond to any of the mitogens.

No significant differences were seen between the exposed and sham-exposed rats in either the percentages or the total numbers of B- and T-cells per spleen.

Significant differences were not detected between the exposed and sham-exposed rats in the percentage and total number of CRPC per spleen.

In the animals immunized with SRBC, the exposed animals showed a slight but statistically insignificant decrease in plaques per spleen, compared with the sham-exposed animals. This test also indicated no grossly significant impairment of the reticuloendothelial system, which first processes the SRBC antigen, and no deficiency in the B-cells' ability to produce antibodies in the presence of T-cells, as the SRBC antigen is T-cell dependent. The exposed and sham-exposed animals had no significant difference in total number of lymphocytes.

COMPARISON AND DISCUSSION

The immunological evaluation phase of the RFR bioeffects program was devoted to screening for possible microwave-induced immunological effects in animals available from the interim (13-month exposure) and terminal (25-month exposure) kills. Multiple immunological tests were conducted to evaluate (1) splenic lymphocyte populations with respect to total number of cells and to numbers of B- and T-cell antigen-positive lymphocytes, and the presence of complement-receptor-positive lymphocytes; (2) the responses of splenic lymphocytes to the following mitogens: phytohemaglutinin (PHA), concanavalin A (ConA), pokeweed (PWM), lipopolysaccharide (LPS), and purified derivative of tuberculin (PPD); and (3) by means of the direct plaque-forming cell assay, the ability of splenic cells to produce antibodies following immunization against the T-cell-dependent antigen sheep red blood cells (SRBC).

In these experiments, 14- and 26-month-old male rats of the strain CAMM(SD)BR were subjected to the same duration and level of RFR exposure for evaluation. The rats were exposed in circular waveguides, and the exposed and sham-exposed animals were handled identically. Care was taken to maintain constant temperature and humidity in the rooms in which all rats were maintained. Barrier procedures were maintained in these rooms so as to keep them pathogen-free. A monitoring program confirmed that the animals were indeed free of bacterial and viral pathogens that could produce artifactual immunological effects.

After 13 months of RFR exposure, the exposed experimental animals had a significant increase in both splenic B- and T-cells when compared with the sham-exposed group. This apparent general stimulation of the lymphoid system in the RFR-exposed animals was not detected in the animals evaluated after 25 months of RFR exposure. Comparison of the exposed and sham-exposed rats in the terminal kills did not reveal any significant differences in the percentage or total numbers of B- and T-cells per spleen. The lack of a significant difference in the terminal-kill animals may be the result of age and the onset of immunoscenescence.

No significant differences were seen between the exposed and sham-exposed rats in the percentage of complement-receptor-positive cells in the spleen at either the interim (13-month exposure) or terminal (25-month exposure) kills. This indicates no difference between the treatment groups in the maturation of lymphocytes as indicated by this procedure.

The plaque assay performed on animals immunized with SRBC in the 13-month exposure group revealed a slight but statistically insignificant increase in plaques per spleen for the exposed animals relative to the sham exposed. This trend appeared reversed in the 25-month exposure group where the exposed animals showed a slight but statistically insignificant decrease in plaques per spleen, compared with the sham-exposed animals. This assay indicated no statistically significant alteration of the reticuloendothelial system, which first processes the SRBC antigen, and no deficiency in the B-cells' ability to produce antibodies in the presence of T-cells, as the SRBC antigen is T-cell dependent.

The mitogen-stimulation studies following 13 months of exposure revealed a significant difference between groups in their responses to various B- and T-cell specific mitogens. A nonsignificant increase in response to PHA and a significant increase in response to LPS and PWM was detected in the RFR-exposed animals. The exposed animals also had a significantly increased response to ConA and a decreased response to PPD ($p = .01$) as compared to the sham-exposed animals. These results suggest a selective effect of RFR on the response of the lymphoreticular system to mitogen stimulation. Mitogen response data was not available from the 25-month exposure studies because the lymphocyte cultures failed to grow.

REFERENCES

Ainti, F., J.C. Cerottini, R.A. Coombs, M. Cooper, H.B. Dickler, S. Freland, H.H. Fudenberg, M.F. Greaves, H.M. Grey, H.G. Kunkel, J. Natvig, J.L. Preud'homme, E. Rabellino, R.E. Ritts, D.S. Rowe, M. Seligman, F.P. Siegal, J. Stjernsward, W.D. Terry, and J. Wybron. Identification, enumeration and isolation of "B" and "T" lymphocytes from human peripheral blood. Clin Immunol Immunopathol 3:584-597 (1975).

Balch, C.M., and J.D. Feldman. Thymus-dependent (T) lymphocytes in the rat. J Immunol 112:79-86 (1974).

Basten, A., J. Miller, J. Sprent, and J. Pye. A receptor for antibody on B lymphocytes. J Exp Med 135:610-626 (1972).

Bianco, C., R. Patrick, and V. Mussenzweig. A population of lymphocytes bearing a membrane receptor for antigen-antibody-complement complexes. J Exp Med 132:702-720 (1970).

Bloom, B.R., and J.R. David. In vitro methods of cell-mediated and tumor immunity. New York: Academic Press, 1976.

Bollinger, J.N., R.L. Lawson, and W.C. Dolle. Research on biological effects of VLF band electromagnetic radiation. SAM-TR-74-52, Nov 1974.

Bowhill, S.A. (Ed). Review of radio science, 1978-1980. International Union of Radio Science (URSI), Brussels, Belgium, 1981.

Czerski, P., K. Ostrowski, M.L. Shore, C. Silverman, M.J. Suess, and B. Waldeskog. Biological effects and health hazards of microwave radiation. Proceedings of an International Symposium, Warsaw, Poland, Oct 1973, pp. 15-18. Warsaw: Polish Medical Publishers, 1974.

Dresser, D.W., and H.H. Wortis. Use of an antiglobulin serum to detect cells producing antibody with low haemolytic efficiency. Nature 208:859-861 (1965).

Hellstrom, K.E., I. Hellstrom, C.C. Jones, H.J. Garrigues, C.K. Chou, and A.W. Guy. In: Abstracts of Bioelectromagnetics Society, p. 75. Third Annual Conference, Washington, DC, 1981.

Huang, A.T., M.E. Engle, J.A. Elder, J.B. Kinn, and T.R. Ward. The effect of microwave radiation (2450 MHz) on the morphology and chromosomes of lymphocytes. Radio Sci 12(6S):173-177 (1977).

Huang, A.T. Immunologic and hematopoietic alterations by 2450-MHz electromagnetic radiation. Bioelectromagnetics 1(1):77-87 (1980).

Ito, K., and W.J. Schull. On the robustness of the T^2 test in multivariate analysis of variance when variance covariance matrices are not equal. Biometrika 51:71-82 (1964).

Janassy, G., and M.F. Greaves. Lymphocyte activation I: Response of T and B lymphocytes to phytomitogens. Clin Exp Immunol 9:483-498 (1971).

Jerne, N.K., A.A. Nordin, and C. Henry. The agar plaque technique for recognizing antibody-producing cells. In Cell-bound antibodies. Conference of the National Academy of Sciences, National Research Council, Washington, DC. Philadelphia: Wister Institute Press, 1963.

Kunz, L.L., R.B. Johnson, A.W. Guy, and C.K. Chou. Pathological evaluation of rats exposed to pulsed radiofrequency radiation: second year progress report (Part II). Bioelectromagnetics Society, 4th Annual Scientific Session, Los Angeles, CA, 1982.

Liburdy, R.P. Radiofrequency radiation alters the immune system. II: Modulation of in vivo lymphocyte circulation. Radiat Res 83(1):66-73 (1980).

Liburdy, R.P. Radiofrequency radiation alters the immune system: Modulation of T- and B-lymphocyte levels and cell-mediated immunocompetence by hyperthermic radiation. Radiat Res 77:34-46 (1979).

Liddle, C.G., J.P. Putnam, J.S. Ali, J.Y. Lewis, B. Bell, M.W. West, and O.H. Lewter. Alteration of circulating antibody response of mice exposed to 9-GHz pulsed microwaves. Bioelectromagnetics 1:397-404 (1980).

McRee, D.I., R. Faith, E.E. McConnell, and A.W. Guy. Long-term 2450-MHz CW microwave irradiation of rabbits: Evaluation of hematological and immunological effects. J Microwave Power 15(1):45-52 (1980).

Prince, J.E., L.H. Mori, J.W. Fraiser, and J.C. Mitchell. Cytological aspects of RF radiation in the monkey. Aerospace Med 43:759-761 (1972).

Roszkowski, W., S. Szmigielski, M. Janiak, and J.K. Wrembel. Effect of moderate and intensive hyperthermia on spleen, lymph-node and thymus-derived murine lymphocytes in vitro. Immunobiology 156:429-440 (1979).

Roszkowski, W., J.K. Wremble, K. Roszkowski, M. Janiak, and S. Szmigielski. Does whole-body hyperthermia therapy involve participation of the immune system? Int J Cancer 25(2):289-292 (1980).

Schlagel, C.J., K. Sulek, H.S. Ho, W.M. Leach, A. Ahmed, and J.N. Woody. Biologic effects of microwave exposure. II. Studies on the mechanisms controlling susceptibility to microwave-induced increases in complement-receptor-positive spleen cells. Bioelectromagnetics 1:405-414 (1980).

Smialowicz, R.J., J.S. Ali, E. Berman, S.J. Bursian, J.B. Kinn, C.G. Liddle, L.W. Reiter, C.W. Weil. Chronic exposure of rats to 100-MHz (CW) radiofrequency radiation: Assessment of biological effects. Radiat Res 86:488-505 (1981).

Smialowicz, R.J., C.M. Weil, J.B. Kinn, and J.A. Elder. Exposure of rats to 425-MHz (CW) microwave radiation: Effects on lymphocytes. J Microwave Power 17(3):211-221 (1982a).

Smialowicz, R.J., M.M. Riddle, R.R. Rogers, and G.A. Stott. Assessment of immune function development in mice irradiated in utero with 2450-MHz microwaves. J Microwave Power 17(2):121-126 (1982b).

Smialowicz, R.J. Hematologic and immunologic effects of nonionizing electromagnetic radiation. Bull NY Acad Med Sci 55:1094-1118 (1979).

Sulek, K., C.J. Schlagel, W. Wiktor-Jedrzejczak, H.S. Ho, W.M. Leach, A. Ahmed, and J.N. Woody. Biologic effects of microwave exposure. I. Threshold conditions for the induction of the increase in complement-receptor-positive (CR+) mouse spleen cells following exposure to 2450-MHz microwaves. Radiat Res 83:127 (1980).

Wiktor-Jedrzejczak, W., A. Ahmed, P. Czerski, W.M. Leach, and K.W. Sell. Effect of microwaves (2450 MHz) on the immune system in mice: Studies of nucleic acid and protein synthesis. Bioelectromagnetics 1:161-170 (1980).

Wiktor-Jedrzejczak W., A. Ahmed, P. Czerski, W.M. Leach, and K.W. Sell. Immune response of mice to 2450 MHz microwave radiation: Overview of immunology and empirical studies of lymphoid splenic cells. Radio Sci 12(6S):209-219 (1977).

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