TABLE 8. ANALYSIS OF CELLULAR COMPOSITION IN BONE MARROW AND SPLEEN OF IRRADIATED AND CONTROL ANIMALS BY FLOW CYTOMETRY AND SPECIFIC ANTIBODIES DEFINING B AND T LYMPHOCYTES

	Chi-squared (1) (p value) 12 Months			
	B Cells		T Cells	
Variable analyzed	Marrow	Spleen	Marrow	Spleen
Percentage of entire population bearing antigen	1.782 (0.182)	0.633 (0.426)	4.248 (0.039)	0.098 (0.754)
Percentage of low light scatter bearing antigen	0.578 (0.447)	0.081 (0.775)	1.337 (0.248)	0.023 (0.879)
Percentage of high light scatter bearing antigen	0.921 (0.337)	3.755 (0.053)	0.613 (0.434)	1.485 (0.223)
Mean expression of antigen over entire population	0.400 (0.527)	2.996 (0.083)	1.537 (0.215)	0.215 (0.643)
Mean expression of antigen in low-light-scatter population	0.395 (0.530)	5.684 (0.017)	2.208 (0.137)	0.198 (0.656)
Mean expression of antigen in high-light-scatter population	0.411 (0.522)	17.134 (0.000)	0.620 (0.431)	1.319 (0.251)

TABLE 9. FLOW CYTOMETRIC ANALYSIS OF CELLULAR COMPOSITION OF BONE MARROW, SPLEEN, AND THYMUS OF IRRADIATED AND CONTROL ANIMALS, USING FORWARD-ANGLE (CELL SIZE) AND RIGHT-ANGLE (CELL COMPLEXITY) PARAMETERS

	Chi-squared (1) (p value)					
	6 Months			12 Months		
Variable analyzed	Marrow	Spleen	Thymus	Marrow	Spleen	Thymus
Total nucleated cells per organ x 10^6	0.032 (0.857)	0.232 (0.630)	1.377 (0.241)	ND	ND	ND
Cell count per mg x 10^6	ND (0.739)	0.111 (0.358)	0.844	ND (0.675)	0.175 (0.236)	1.403
Percentage of cells with low light scatter	5.522 (0.019)	0.017 (0.896)	0.380 (0.537)	1.549 (0.213)	0.218 (0.641)	0.122 (0.727)
Percentage of cells with medium light scatter	2.673 (0.102)	ND	ND	ND	ND	ND
Percentage of cells with high light scatter	2.263 (0.133)	0.541 (0.462)	0.009 (0.923)	1.961 (0.161)	0.083 (0.773)	ND

Discussion

Significant issues dealing with the effects of low-level chronic exposure of laboratory animals to RF radiation have remained unresolved. Alterations in the hematopoietic and immunologic networks have been reported in animals exposed to RF at and below 10 mW/cm^2, but these effects could be due to thermal changes within the animals. The experiments reported here demonstrate alterations in the hematopoietic and immunologic systems of rats after long-term (6 and 12 mo) exposures to very low levels of RFR (SAR, 0.15-0.4 W/kg; .48 mW/cm^2). Effects of the 6-mo exposure include (a) an increased number of marrow hematopoietic progenitor cells (CFU-C); (b) increased proliferative responses to PPD by splenic B cells; (c) decreased total B lymphocytes in the marrow; (d) decreased proportion of low light scatter, i.e., small cells in marrow. The following effects are associated with the 12-mo exposure to RFR: (a) increased number of hematopoietic precursors; (b) reduction in cell-surface expression of Thy 1.1 surface antigen on thymocytes; (c) reduction in the mean cell-surface density of s-Ig on small lymphocytes (low light scatter) in the spleen. Some of these seven significant group differences may be statistical artifacts since 70 separate tests were done at the .05 level on animals exposed to RFR for either 6 or 12 mo. Even if the null hypothesis of no group difference were true, we would expect to find, on the average, 3.5 statistically significant differences after both the 6- and 12-mo exposures.

In contrast to a previous study at this institution, among the rats exposed for 6 mo we observed marginally increased spleen cell response to the B-cell mitogen PPD and no increased proliferative response to LPS, PWM, or Con A. Relative to days 2-5, PPD control responses were extremely low on day 1 for both exposed and nonexposed rats. Stimulated values for day 1 were within the range of values for assays on the other days. The ln (stimulation index) ratio is sensitive to this type of data anomaly. When day 1 was excluded from analysis, the mean responses for the two groups were marginally significantly different in the opposite direction; i.e., the mean ln (PPD/PPD control) response of the exposed group was lower than that of the nonexposed ($X^2(1) = 3.371$, $p = .066$). Since the PPD response showed no significant differences between the groups in the observed scale,

either including or excluding day 1, we believe this particular result may be spurious. In the LPS assay, stimulated responses were actually lower than the medium control values for one exposed rat assayed on day 2 and three nonexposed rats assayed on day 3. When those four observations were excluded from analysis, (1) the exposed animals exhibited a significantly lower response to LPS in the observed scale than did the nonexposed (X^2 = 4.637, p = 0.31), and (2) no significant difference was observed for LPS in the ln scale.

In contrast to our previous study where microscopic quantitation of T and B cells in the spleens of exposed and sham-exposed rats was used, the flow cytometric approach (where 10,000 cells were evaluated) revealed no group alterations in the number and frequency of T or B cells. However, the mean expression of s-Ig was reduced on splenic B cells after 12 mo of RFR exposure.

Six months of exposure (but not 12 mo) produced a selective decrease in B cells in the marrow of exposed rats. Several explanations for such a change are possible: (1) the rate of primary B-cell production in the marrow may be decreased due to the decreased cycling of and/or numbers of B-cell precursors; (b) the rate of recirculation of B cells to the marrow may be lower; or (c) the release of newly born B cells from the marrow to the blood may be faster. Our data do not permit us to select one interpretation at this time. At the same time, the frequency of small (low light scatter) cells in the marrow of exposed rats was diminished. Low-light-scatter cells encompass small lymphoid cells and immature erythroid elements and noncycling stem cells.

The most surprising and consistent finding of both exposure periods was the increased number of hematopoietic progenitor cells (CFL-C) in the marrow of exposed rats. Increased CFU-C can be explained by a decreased survival of mature monocytes, macrophages, and granulocytes in the peripheral lymphoid tissues, which necessitates a compensatory increase in their progenitor-cell activity. Alternatively, RFR may have a direct effect on the progenitor cells, increasing their production rate. Finally, RFR may cause increased production of growth stimulatory hormones by macrophages, lung cells, and T cells; such hormones may then expand the pool of hematopoietic progenitors in the marrow.

Animal Health Profile

Many research projects to assess the biological effects and health hazards of microwave radiation have involved single or limited biological endpoint evaluations. During the last few years a health profile has proven more useful to researchers for diagnosing and understanding abnormalities in their experimental animals. Several advantages are inherent in the health profile evaluation of experimental rats in this study. The profile helps to uncover unsuspected organ-system malfunction. In animals with subclinical or undiagnosed abnormalities, the profile can aid in selecting appropriate tests to define the animal's problem. This allows emphasis to be placed on the current interpretation of profile results and the interrelation of different test results rather than on individual test selection. The health profile permits a better understanding of the pathophysiology of abnormal or disease states. As an organ system abnormality or disease changes, the blood chemistry and hematology can be monitored. The profile can demonstrate multisystemic organ involvement, often missed if only individual tests are performed. The profile test results may indicate a specific condition that requires other tests to assess the full significance of a particular abnormality.

During the exposure period the rats were bled every 6 wk, with the first bleeding during the 7th wk of exposure. This duplicates the experimental stress that animals in the original project were subjected to. The retro-orbital technique was used for bloodletting. At the first bleeding session 100 blood samples were tested for the corticosterone level. The serum collected from all other periodic bleedings was not analyzed other than to be retained as a pooled control for the serum chemistry evaluations. Hematological, corticosterone, thyroxine (T_4), protein electrophoresis, and serum chemistry evaluations were made on blood collected during the interim sacrifices at 6 and 12 mo of exposure.

The serum chemistry evaluation consists of a panel of 24 parameters from each sample, incuding various electrolytes, enzymes, and blood gases. This panel provides a multiphasic evaluation of the animals' physiological systems.

Although not a method for determining specific proteins, serum protein electrophoresis is considered valuable in organ panels or health profiles. It is the single most sensitive procedure for detecting monoclonal gammopathies and is used to determine certain proteins such as albumin and immunoglobulins. With a well-resolved system, even small monoclonal or oligoclonal bands can be easily identified, giving evidence of intense immunologic stimulation such as may accompany serious viral infections or tissue necrosis.

The hematological data are seldom diagnostic as individual tests but are invaluable as an adjunct to the health profile. They indicate the presence or absence of systemic stress, inflammatory disease, bone marrow disease, and neoplasia. This information may help in formulating a presumptive or final diagnosis or in monitoring a physiological or disease abnormality that may affect the immunological status of the animal and lead to invalid immunological test results.

Pituitary-adrenal axis activity as indexed by plasma corticosterone levels has long been interpreted as an indicator of general arousal, i.e., anxiety, fear, or stress. Stress can be attributed to very specific, noxious stimuli or to a diffuse, nonspecific stimulus complex, either physical or psychological in nature (Selye, 1950; Levine, 1972). Exposure to microwave radiation under various frequencies and power levels produces alterations in pituitary-adrenal activity (Lu et al., 1980), albeit at levels substantially higher than used in this study.

The assessment of plasma corticosterone was originally intended solely as a check on the stress-free nature of the experimental housing and protocol in compliance with the original statement of work. Of concern was the possibility that a stressful environment might result in artificial microwave effects or mask important microwave bioeffects. This endpoint also provides additional information about animal well-being prior to assessment of immunological competence.

Methods

Serum Chemistries. The blood for the serum chemistries was collected in B & D microtainer serum-separator tubes. The serum was separated and then refrigerated in separate glass vials until it was analyzed later that day on a Technicon SMAC computer-controlled biochemical analyzer. The analysis done on the SMAC analyzer for this project was directly supervised by a board-certified veterinary pathologist. The following test values were obtained by the SMAC analyzer:

1. Glucose
2. Urea nitrogen (BUN)
3. Creatinine
4. Sodium
5. Potassium
6. Chloride
7. Carbon dioxide
8. Uric acid
9. Total bilirubin
10. Direct bilirubin
11. Ionized calcium
12. Calcium
13. Phosphorus
14. Alkaline phosphatase
15. Lactate dehydrogenase (LDH)
16. Serum glutamic-oxaloacetic transaminase (SGOT)
17. Serum glutamic-pyruvic transaminase (SGPT)
18. Cholesterol
19. Triglycerides
20. Total protein
21. Albumin
22. Globulin

Thyroxine. The radioimmunoassay for T_4 is extremely sensitive, and small quantities of serum are required for the test. In the test procedure, serum of the 20 rats was mixed with radioactivity-labeled thyroxine (^{125}I) and 8 anilinolnapthalene sulfonic acid (ANS), then an immobilized T_4 antiserum was added and the mixture was incubated at room temperature. The ANS displaces the T_4 from the serum proteins. During incubation the displaced T_4 competes with the labeled T_4 for the immobilized T_4 antibodies on the basis of their relative concentrations.

The amount of labeled T_4 that binds with the antibody is inversely related to the amount of unlabeled endogenous T_4 present in the serum. After incubation the mixture was centrifuged and the immobilized T_4-antibody complex was concentrated at the bottom of the tube in the form of a pellet. The unbound T_4 in the supernatant was decanted, and the radioactivity associated with the pellet was counted. A standard curve prepared with precalibrated T_4 standards was used to determine the concentration of T_4 in the rat serum.

The procedure was a modified quantimmune T_4 radioimmunoassay (RIA) method based on the principles of RIA described by Berson and Yalow (1960). A Packard autogamma scintillation spectrometer 5130 was used.

Protein Electrophoresis. A Sepratek system (Gelman Instrument Co., Ann Arbor, MI) was used in the protein electrophoresis analysis. Eight serum samples (0.25 µl each) were applied with the eight-place applicator to a single cellulose acetate membrane (5.7 x 12.7 mm). Three of these membranes were mounted in a single electrophoresis chamber containing 700 ml buffer (tris-barbital-sodium barbital pH 8.8). The samples were electrophoresed for 60 min at 300 V, then the membranes were stained with Ponceau S solution, clarified, and mounted on glass slides. This produced well-defined serum protein fractions on a transparent background. Quantitation with a digital computing densitometer provided a scan of the optical density at 525 nm and computed the area under the portions of the scan corresponding to the various protein fractions.

Included in each batch of samples were several control serums. These consisted of commercially obtained pure albumin, beta globulin, and gamma globulin dissolved in saline. These controls assisted in locating the various fractions on the sample densitometer scan and provided a way to check the integration accuracy of the densitometer.

Hematology. The hematological tests followed standard laboratory practice. The hematological parameters, determined on a minimum of 0.3 ml of blood in EDTA, were

Hemoglobin	Packed cell volume (PCV)
White blood count (WBC)	Indices
Red blood count (RBC)	Differential count

A Coulter counter was used for the WBC and RBC; the hemoglobin was determined by the cyanomethemoglobin method, and trained hematology technicians did the differential counts. The blood film was prepared on a slide from a thoroughly mixed sample, and the slide was stained by Wright's method and evaluated microscopically. The work of the hematology technicians was supervised and reviewed by a board-certified veterinary pathologist. The data were compared with reference data from the literature and from the Laboratory Animal Data Bank.

Corticosterone Assay. The microfluorometric assay procedure used to analyze plasma corticosterone was based on a procedure described by Glick et al. (1964), and modified by Riley and Spackman (1976), for use on small samples (50 μl) obtained from mice. The technique was further modified to fit the needs of the original RFR exposure study on rats, using the retro-orbital bleeding technique. After correction for the presence of nonspecific fluorogens, the method gives accurate results for quantities of corticosterone as low as 10 ng/ml.

Results

Table 10 lists the values of the serum chemistries and T_4 for 6- and 12-mo exposures. Individual t-tests did not show any significant effect ($p > 0.05$) between the exposed and sham-exposed groups. Table 11 shows the 6- and 12-mo results of the protein electrophoresis. The gamma globulin showed a marginal effect ($p = 0.0477$, $df = 37$) at 6 mo but not at 12. To compare with the results from the original study, no effect had been observed during the 15 sampling sessions over the 25-mo exposure; therefore, the gamma globulin effect could be due to the variation of the data. The hematology data are shown in Table 12. No significant effect was found in any parameter.

Figure 11 shows the corticosterone level in rats exposed for 6 wk, 6 mo, and 12 mo. The unit is expressed as "micrograms per 100 milliliters" instead of "nanogram per milliliter" so that these values can be more easily compared with those reported in the literature. Statistical evaluation using T-test on these three observations failed to detect any significant difference ($p > 0.05$).

At the first sampling (at 6 wk of exposure) of the original long-term study, the corticosterone level showed a significant difference ($t = 2.06$, $p = .04$, $df = 154$). This effect was not replicated in the present study, so the previously observed effect was most likely a random variation. Inspection of the individual data indicates that data variance was large regardless of the treatment condition--probably related to variations in time from removing an animal from the waveguide cages to the blood sampling. Riley and Spackman (1976) showed that plasma corticosterone increased substantially after 4 min of animal handling. In our study, blood sampling was done within 1 min after the animal was removed from the holding bin. The daily handling procedure--removing the rat from the waveguide cage and weighing and placing it in its holding bin--occurred during 0800-0805 for the group of eight rats to be killed. The necropsy took place during 0830-0850, so the time between the animal's first being touched and the blood sampling was about 30-50 min; however, because of its daily nature the handling stress should have been mild.

TABLE 10. SERUM CHEMISTRY AND THYROXINE DATA OF RATS EXPOSED/SHAM EXPOSED FOR 6 OR 12 MONTHS

Parameter	Exp	Mean	SE	Max	Min
		6 months (N=20)			
Glucose	E	125.5	3.08	153	98
(mg/dl)	S	126.7	2.99	156	104
BUN	E	20.2	0.41	23	16
(mg/dl)	S	20.0	0.39	24	18
Creatinine	E	0.51	0.018	0.6	0.3
(mg/dl)	S	0.53	0.015	0.6	0.4
Sodium	E	144.3	0.35	147	141
(meq/dl)	S	143.9	0.46	146	138
Potassium	E	6.18	0.20	8.9	4.9
(meq/dl)	S	6.11	0.21	8.0	4.8
Chloride	E	102.1	0.27	104	100
(meq/dl)	S	102.2	0.39	106	99
Carbon Dioxide	E	26.9	0.58	32	23
(meq/dl)	S	26.6	0.53	30	20
Uric Acid	E	1.52	0.08	2.2	0.9
(mg/dl)	S	1.64	0.12	3.1	1.0
Total Bilirubin	E	0.05	0.01	0.2	0.0
(mg/dl)	S	0.08	0.01	0.1	0.0
Ionized Calcium	E	4.77	0.11	6.7	4.4
(mg/dl)	S	4.77	0.10	6.5	4.3
Calcium	E	10.1	0.24	14.4	9.3
(mg/dl)	S	9.95	0.19	13.4	9.3
Phosphorus	E	5.36	0.10	6.1	4.4
(mg/dl)	S	5.28	0.11	6.1	4.4
Alkaline Phosphatase	E	159.3	6.17	246	116
(U/l)	S	160.2	3.20	193	131
LDH	E	692.8	58.2	1157	252
(U/l)	S	792.4	61.9	1633	405
SGOT	E	123.6	6.29	177	70
(U/l)	S	132.2	9.53	282	82
SGPT	E	42.7	1.45	60	33
(U/l)	S	39.9	1.53	60	31
Cholesterol	E	106.8	5.70	165	49
(mg/dl)	S	97.9	3.75	137	64
Triglycerides	E	104.1	8.71	220	46
(mg/dl)	S	103.1	7.71	173	55
Total Protein	E	6.21	0.06	6.7	5.7
(g/dl)	S	6.08	0.05	6.6	5.8
Albumin	E	3.43	0.04	3.8	3.1
(g/dl)	S	3.43	0.03	3.6	3.2
Globulin	E	2.78	0.06	3.1	2.4
(g/dl)	S	2.66	0.05	3.2	2.2
Albumin/Globulin	E	1.26	0.03	1.5	1.0
	S	1.31	0.03	1.6	1.1
T4	E	3.48	0.14	4.9	2.4
(μg/dl)	S	3.57	0.14	4.9	2.2

TABLE 10. (continued)

Parameter	Exp	Mean	SE	Max	Min
		12 months (N=20)			
Glucose	E	124.4	3.74	150	85
	S	125.7	3.35	150	100
BUN	E	17.8	0.35	23	16
	S	18.5	0.64	29	16
Creatinine	E	0.64	0.02	0.8	0.5
	S	0.71	0.05	1.2	0.5
Sodium	E	143.7	0.40	148	140
	S	144.8	0.60	150	140
Potassium	E	5.86	0.22	8.0	4.9
	S	5.81	0.18	7.5	4.8
Chloride	E	102.4	0.33	105	98
	S	102.7	0.55	107	98
Carbon Dioxide	E	27.1	0.46	30	23
	S	28.0	0.54	31	22
Uric Acid	E	1.52	0.08	2.5	1.0
	S	1.40	0.07	2.1	1.0
Total Bilirubin	E	0.11	0.02	0.2	0
	S	0.14	0.02	0.3	0
Ionized Calcium	E	4.77	0.04	5.0	4.4
	S	4.78	0.03	4.9	4.4
Calcium	E	10.1	0.07	10.6	9.4
	S	10.1	0.08	10.5	9.0
Phosphorus	E	5.29	0.06	5.7	4.6
	S	5.44	0.07	6.2	4.9
Alkaline Phosphatase	E	168.9	6.54	248	130
	S	166.6	7.63	236	114
LDH	E	723.6	66.7	1148	358
	S	667.0	51.9	1098	332
SGOT	E	138.8	8.80	7	79
	S	129.9	7.69	1	69
SGPT	E	48.2	3.49	89	31
	S	47.0	2.78	74	31
Cholesterol	E	196.7	12.3	314	114
	S	189.2	12.4	314	121
Triglycerides	E	187.6	16.0	328	96
	S	160.9	12.9	290	78
Total Protein	E	6.21	0.05	6.4	5.8
	S	6.18	0.06	6.8	5.8
Albumin	E	3.27	0.04	3.5	2.9
	S	3.27	0.05	3.6	2.7
Globulin	E	2.94	0.04	3.2	2.6
	S	2.92	0.05	3.3	2.4
Albumin/Globulin	E	1.12	0.02	1.3	0.9
	S	1.12	0.03	1.4	0.8
T4	E	4.02	0.24	6.2	2.6
	S	4.01	0.24	6.7	2.2

TABLE 11. PROTEIN ELECTROPHORESIS DATA OF RATS EXPOSED/SHAM-EXPOSED FOR 6 OR 12 MONTHS

	EXP	N	Mean	SE	Max	Min
			6 months			
Albumin	E	20	49.6	2.60	62	4.5
(%)	S	19	51.6	0.66	57	48
Alpha 1	E	20	19.6	1.33	33	2.5
(%)	S	19	21.0	0.69	26	15
Alpha 2	E	20	7.65	0.23	9	5
(%)	S	19	8.26	0.31	11	6
Beta	E	20	15.7	0.25	18	14
(%)	S	19	15.8	0.26	18	14
Gamma	E	20	4.80*	0.65	15	2
(%)	S	19	3.32*	0.29	6	2
			12 months			
Albumin	E	20	55.0	1.49	69	46
	S	20	53.9	1.70	69	35
Alpha 1	E	20	18.3	1.41	27	7
	S	20	19.6	1.72	38	5
Alpha 2	E	20	8.30	0.38	12	5
	S	20	8.40	0.27	11	6
Beta	E	20	6.15	0.46	11	3
	S	20	5.35	0.44	9	2
Gamma	E	20	12.5	0.47	16	9
	S	20	13.0	0.49	17	9

* $p < 0.05$

TABLE 12. HEMATOLOGY DATA OF RATS EXPOSED/SHAM-EXPOSED FOR 6 OR 12 MONTHS

	EXP	N	Mean	SE	Max	Min
				6 months		
WBC	E	20	6.40	0.39	8.8	0.3
	S	20	6.97	0.19	8.7	5.5
RBC	E	20	8.03	0.06	8.7	7.6
	S	20	8.01	0.05	8.4	7.6
HGB	E	20	15.5	0.09	16.4	14.6
	S	20	15.4	0.07	15.8	14.7
HCT	E	20	43.7	0.43	46.9	40.0
	S	20	43.5	0.32	45.7	40.3
MCV	E	20	54.4	0.39	58	51
	S	20	54.3	0.31	57	52
MCH	E	20	19.3	0.10	20.2	18.2
	S	20	18.9	0.39	20.0	11.9
MCHC	E	20	35.5	0.21	36.8	33.8
	S	20	35.4	0.20	37.7	34.4
Lymphocytes	E	20	69.4	4.76	87	6
	S	20	76.0	4.09	92	8
Mono	E	14	3.14	0.54	8	1
	S	15	2.53	0.35	4	1
Eosin	E	17	2.82	0.52	9	1
	S	12	2.58	0.48	6	1
				12 months		
WBC	E	20	4.64	0.30	7.0	2.6
	S	20	4.99	0.18	6.4	3.6
RBC	E	20	7.69	0.08	8.4	7.0
	S	20	7.59	0.09	8.0	6.9
HGB	E	20	15.1	0.11	16.0	14.5
	S	20	15.2	0.14	16.1	13.9
HCT	E	20	44.2	0.87	51.0	37.5
	S	20	43.6	0.90	49.6	35.4
MCV	E	20	57.4	0.72	61.0	51.0
	S	20	57.3	0.74	63.0	51.3
MCH	E	20	19.7	0.15	20.8	18.7
	S	20	20.0	0.15	21.3	18.6
MCHC	E	20	34.5	0.62	39.8	31.4
	S	20	35.0	0.60	39.4	29.6
Neutrophil	E	20	1.31	0.14	2.59	0.38
	S	20	1.53	0.17	3.52	0.69
Lymphocyte	E	20	3.12	0.24	5.24	1.58
	S	20	3.17	0.24	5.16	0.13
Mono	E	20	130.9	38.1	748	0
	S	20	90.0	25.6	495	0
Eosin	E	20	55.3	12.4	162	0
	S	20	44.3	12.5	196	0

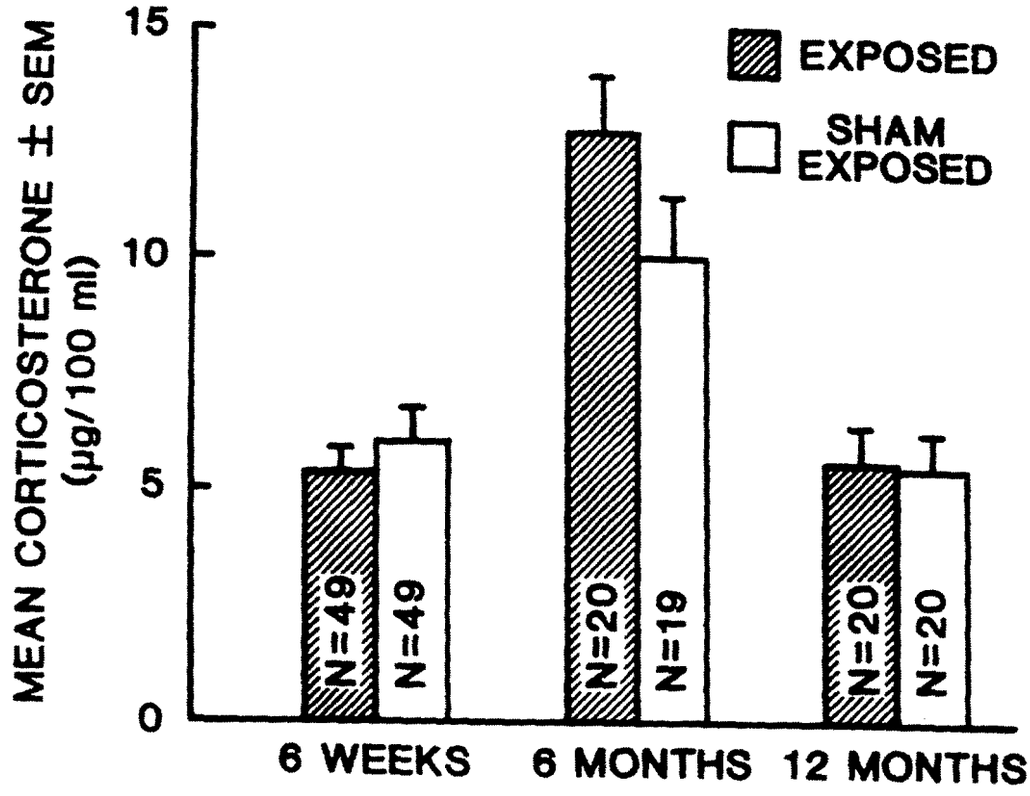

Figure 11. Corticosterone levels in rats exposed for 6 wk, 6 mo, and 12 mo.

Gross Pathological and Histopathological Evaluation

During this experiment all animals were histologically evaluated at death. Documentation of morphological lesions helps to provide a definitive diagnosis of any organ system abnormally present and validates the immune competency studies.

A comparison of spontaneous and age-related lesions in the exposed and sham-exposed animals in this study and in animals of the previous project will help expand the data base on the effects of long-term low-level RFR.

Methods

Animals that died spontaneously were necropsied and the tissues fixed as rapidly as possible. Prior to euthanasia the final-sacrifice animals were examined for any clinical evidence of disease or deviation from normal in physical appearance and behavior; any defects were described and classified at that time. At necropsy, living rats were anesthetized in a halothaneoxygen chamber and euthanized by rapid exsanguination via the carotid or brachial arteries or both. This method minimizes anoxic or agonal hemorrhages and hypostatic congestion. It also allows collecting the maximum volume of blood for serum chemistry and immune system evaluation. If an animal died spontaneously, the clotted heart blood was collected and the serum extracted for possible serology. The necropsies followed a rigid protocol and were done by a veterinary pathologist or a trained research technician under the pathologist's supervision.

Results

Histopathological results for the 6- and 12-mo exposures are listed in Table 13. After the 6-mo exposure, 75 lesions were observed in the exposed group and 62 in the sham-exposed. The 12-mo group had 82 lesions in exposed rats and 75 in controls. Statistical analysis of the total or individual lesions shows no difference between the two groups for both 6- and 12-mo exposure, except peribronchiolar lymphoretical proliferation did increase significantly in 12-mo exposed rats (comparison of binomial proportions, $p = 0.0046$). In general, the occurrence of peribronchiolar

lymphoreticular proliferation was high in both exposed and sham-exposed groups. Six neoplastic lesions (5 benign, 1 malignant) were found in the 12-mo animals. The occurrence was too low to make any statistical comparison meaningful.

TABLE 13. HISTOPATHOLOGICAL RESULTS OF 6- AND 12-MONTH EXPOSURES

Lesion	6 Months		12 Months	
	Exp	SE	Exp	SE
Adrenal				
adenoma, cortical				1
cellular alteration	7	4	12	8
hyperplasia			2	4
Aorta				
medial calcification			1	
Colon				
subacute nonsuppurative colitis				1
Epididymus				
sperm granuloma		1		
Esophagus				
megaesophagus	1			
Heart				
antoni type a neuronoma			1	1
atrioventricular valves, myxofibromatous degeneration	2	2		
cardiomyopathy	1	1	3	3
cartilaginous foci, subaortic valve	2	1	6	5
fibrosis, tricuspid valve				1
foci of cartilage, base of aortic valve	5	3	1	
myocarditis, multifocal			1	1
Inguinal lymph node				
abscess		1		
Kidney				
chronic glomerulonephropathy	5	4	13	11
chronic interstitial nephritis		3		
cystic tubules	4	5		
Liver				
mononuclear cell infiltration	2			
microfocal granuloma, single			1	
single foci of chronic hepatitis		1		
Lung				
blood inhalation			1	2
peribronchiolar lymphoreticular proliferation	20	20	19	10
petechial hemorrhages		1		
granulomatous inhalation pneumonia	1			
Mandibular sg				
nonsuppurative periductal sialoadenitis	1			
periductal lymphoreticular infiltration		1		

TABLE 13. (continued)

Lesion	6 Months Exp	6 Months SE	12 Months Exp	12 Months SE
Pancreas				
chronic focal pancreatitis				1
ductal lithiasis				2
eyelet cell adenoma				1
Parotid				
nonsuppurative periductal adenitis			1	
periductal lymphoreticular proliferation		1		
Pituitary				
cyst	5	3		
Preputial gland				
abscess	2	1	3	3
cystic pyogranulomatous adenitis			1	3
chronic adenitis	2	1		
cystic hyperplasia	2			1
Prostate				
chronic nonsuppurative prostatitis	1	2		
granulomatous prostatitis	1			
keratin cyst			1	
nonsuppurative adenitis				1
Right testicle				
atrophy, tubular				1
Salivary gland				
periductal adenitis	4	3	2	5
Skin				
pilomatricoma, back, benign				1
Small intestine				
mucoid cystic adenocarcinoma			1	
Stomach				
gastric squamous cell papilloma, diffuse				1
ectopic pancreas in gastric wall	1			
squamous papillomatosis	1			
Testes				
bilateral hypoplasia		1		
dystrophic seminiferous tubular mineralization	1			
Thyroid				
atrophy, diffuse			6	2
hyperplasia, c-cell			1	
follicular squamous metaplasia		1	1	
Trachea				
subacute tracheitis	1			
Zymbals gland				
chronic adenitis	3	1	4	5
Total lesions	75	62	82	75

The pathological results of the original study had indicated that the only effect on nonneoplastic and neoplastic lesions, including both benign and malignant tumors, was a statistically significant increase of the total primary malignant tumors in the exposed animals. Even that occurrence (18 versus 5 in 100 exposed and 100 control animals) was within the normal variation of tumor incidence in chronic studies. The mathematical significant difference is provocative; any biological significance is questionable. After the histopathological examination of the animals in this study, we could not answer the question of tumor incidence because very few tumors developed within the 12 mo of the experiment.

METABOLISM

Two distinct actions of microwave radiation on mammals can, in principle, be distinguished: 1) the hypothesized direct effects on biomolecules and cell structure through as yet undemonstrated mechanisms and 2) the inescapable and demonstrable thermal consequences of the heat produced within biological material during exposure. Although the exposure levels used in the original long-term study were decisively below those known to produce obvious thermal damage to cellular processes, some degree of life prolongation and a reduction in cancer incidence in the exposed population may be a side effect of minor thermal energy accumulated throughout a lifetime of exposure (Sacher, 1977). Such an effect could occur at ambient temperatures below thermoneutrality, depending on how the average male rat metabolizes the thermal energy deposited within it. One option is that it uses the absorbed energy to maintain thermal equilibrium with the ambient environment, as an alternative to using internal chemical stores of energy. This lowered requirement for chemical energy would then result in reduced food intake, oxygen consumption, and total body mass and/or total fat stores and in the paradoxical finding of increased longevity (cf. McCay et al., 1939; Berg and Simms, 1960; Sacher and Duffy, 1978).

No differences between exposed and sham-exposed animals in the long-term study were observed in terms of longevity or in any of the metabolic measurements used, nor for the measures of general health. The data suggest that exposure to microwave radiation at an average lifetime SAR of approximately 0.25 W/kg was so minimal as to be of little metabolic consequence with respect to the total energy balance of the exposed animal population.

Objectives

The above suggestion of minimal impact of a lifetime exposure to microwave radiation lacks the support that would be provided by a set of positive control experiments that demonstrated the reliability and

sensitivity of measurement techniques used. The statement made by the original study will have meaning only within the context of the dose-response relationship of specific endpoints at various microwave exposure levels.

The objective of this phase of the research is to provide an understanding of the dose-response relationships of the health profile and metabolic and immunological parameters assessed in the original study. Additional emphasis will be placed on the metabolic relationships through factorial combination of multiple microwave-exposure levels and ambient temperatures.

Scope of Work

To determine a dose-response relationship for demonstrable microwave effects, we exposed nine groups of 10 rats (90-day-old males, same supplier as before) each to 2450-MHz CW microwave radiation for 6 wk. The nine treatment conditions represent a factorial combination of three microwave power densities and three ambient temperatures. The power densities (5, 10, and 15 mW/cm^2) all exceeded the level used in the original study by at least a factor of 10. The corresponding average SARs were 2.5, 5, and 7.5 W/kg. Ten animals were sham exposed and served as controls at each ambient temperature (17.8, 22.2, and 26.7^{o}C [64, 72, 80°F]). The relative humidity was maintained at 55 $\pm$ 10%.

One of the two SPF animal-exposure rooms was used for this study. The logistics of the exposure facility are such that three exposure levels can be maintained simultaneously in three separate alcoves, with the ambient temperature held constant at one selected level. Therefore, only three actual exposure periods had to be conducted, one at each of the three ambient temperatures. Each alcove contained 10 animals housed in the exposure apparatus, and 10 sham-exposed animals were partitioned between the three alcoves to serve as control subjects for each ambient temperature.

During each exposure period, metabolism data were collected daily from the rats housed in each alcove, including assessment of body weight and food and water consumption. Respiratory gas exchange (i.e., oxygen consumption and carbon dioxide production) was measured simultaneously on four animals in airtight metabolism chambers. Three of these animals were microwave exposed, each at a different power density, and the fourth was sham-exposed. Each animal was in the metabolism chamber for 2 days. During the 6-wk exposure, the metabolic gas exchange was measured twice for all rats.

At death, serum chemistry, protein electrophoresis, T_4, and corticosterone were measured. Also, gross pathological and histopathological examinations were made after sacrifice of animals. As part of the immunological competence studies described before, the spleen, thymus, and bone marrow tissues were removed for testing.

Assessment Rationale

To survive, all mammalian species must maintain internal body temperature within very narrow limits. Much literature details the autonomic mechanisms of thermal regulation as well as the behavioral strategies evolved by each species to cope with their ever-changing thermal environment.

Deposition of thermalizing radiant energy is an inescapable consequence of microwave exposure, particularly at higher power densities. At times bioeffects research literature inadvertently focuses attention on the obvious cellular damage accompanying elevated tissue temperatures, furthering misunderstanding of the less dramatic responses of the irradiated organism. Although an elevated rectal temperature indicates that various regulatory mechanisms have failed to maintain normal thermal equilibrium in response to an externally imposed thermal burden, the finding of a normal rectal temperature (i.e., thermal regulatory mechanisms

have not failed) is often cited as if to indicate that only a "non-thermalizing" level of microwave exposure had been used.

Recently a few reports have appeared describing certain compensatory adjustments effected during microwave exposure, such as reduction in food intake (Gage, 1979; Chou et al., 1983a), reduction in oxygen consumption (Phillips, 1975; Ho and Edwards, 1977), and increased evaporative heat loss (Gordon, 1982). Some researchers have begun to characterize behavioral thermoregulatory strategies used by various species during exposure to microwave radiation, including the lizard (D'Andrea et al., 1978), monkey (deLorge, 1976; Adair and Adams, 1980), and rat (Stern et al., 1979).

Although the behavioral and physiological systems involved in thermoregulation in the various ambient environments are understood in general, no systematic investigation has extended this understanding to the adaptive processes associated with long-term exposure to microwave radiation. The ubiquitous argument concerning thermal versus nonthermal effects demands clarification of the physiological and behavioral strategies mounted in response to microwave energy absorption. Research to clarify these strategies will help us understand how complex thermoregulatory adaptations can affect other physiological systems, thereby producing so-called nonthermal microwave effects.

Ambient temperature is the most effective determinant of thermoregulatory adaptation in animals. Therefore, understanding the thermoregulatory-system response to the interaction of microwave energy deposition at various ambient temperatures is necessary. Thermoneutrality is defined as the ambient temperature range at which the minimum level of metabolic activity is observed, or the TNZ. For the rat this level has been assessed as 28-33°C (Herrington, 1940). At temperatures below the TNZ, metabolism rises due to increased heat production associated with decreasing temperature. At temperatures above the TNZ, metabolism rises due to increased motor activity associated with behavioral thermoregulation. This temperature range of TNZ has been assumed to approximate an index of comfort (Folk, 1966; McNab, 1970; Porter and Gates, 1969).

The classical definition of thermoneutrality has been relaxed somewhat in recent years to include a wider range of behavioral thermoregulatory responses (Bianca, 1974). The usual practice of breeding and maintaining rats within the TNZ has been questioned, and suggestions have been made that a lower ambient temperature (20-25°C) may provide a less stressful and more healthy environment for the laboratory rat (Weihe, 1965; Poole and Stephenson, 1977).

Food intake of the rat varies as a function of ambient temperature; highest intake accompanies ambient temperatures near 0°C and decreases linearly to a minimum at 35-38°C (Brobeck, 1948; Hamilton, 1963; Jakubczak, 1976). Previous studies in this laboratory that varied ambient temperature and monitored food intake in rats exposed to various microwave power densities have found that food intake decreased as an additive function of both rising ambient temperature (21-26°C) and increasing SAR (0.8-7.2 W/kg) (Moe et al., 1976; Lovely et al., 1977).

As ambient temperature rises (but below the TNZ), the rat uses additional physiological and behavioral strategies to thermoregulate. These include dramatic reductions in activity (Hainsworth et al., 1968), initiation of saliva spreading (Hainsworth, 1968), and vasodilation of the tail (Rand et al., 1965; Hellstrom, 1975; Dawson and Keber, 1979).

One outcome of these thermoregulatory adjustments is an increase in the level of stress associated with the environment, as measured by circulating levels of plasma corticosterone. Some investigators have demonstrated that individual rats have varying capacities to deal with thermal stress and implement different strategies to cope with experimentally induced thermal burdens (Robinson et al., 1968; Blackmore, 1970; Hubbard et al., 1976; Isobe et al., 1980). As mentioned in the immunology section, the stress associated with thermoregulatory adjustments may be a determining factor in the alteration of immunological responses. Therefore, plasma corticosterone was assayed on each animal just prior to sacrifice, as an indicator of the perceived demands placed on it by the environment. This information will serve as part of the dose-response data base and help validate the immunological assessments.

Animals from each of the nine experimental conditions were sacrificed and necropsied at the termination of each study. The spleen, thymus, and

bone marrow tissues were removed and analyzed and the data used to form a dose-response analysis of the altered immunological response.

Serum chemistry, hematological, protein-electrophoresis, and T_4 determinations, as well as gross pathological and histological examinations, provide a measure of the general health of each animal prior to sacrifice, for supportive evaluation of the immunological data. Moreover, all these endpoints form part of a larger dose-response evaluation of the endpoints used in the original study.

Methods

Body weight and food and water consumption of each animal were measured daily throughout the course of each study. The method most widely used to determine metabolic rate involves measuring respiratory gas exchange. The use of this measurement as an index of metabolic activity is based on the principle that oxygen is consumed and carbon dioxide is produced in direct proportion to the intensity of metabolic activity. The sum of all metabolic activity within an organism can be measured at the rate at which oxygen is removed from the air in the immediate environment of the organism.

Many technical problems are associated with measuring gas exchange as an index of metabolism. For example, the oxygen analyzers commercially available actually measure the partial pressure of oxygen in the sampled air; therefore the temperature of the air, its relative humidity, the barometric pressure, and the chamber airflow rates all affect the calculation of oxygen consumption. To simplify the gas exchange monitoring system so that it could be routinely performed as part of a standard daily procedure, we chose the equipment for this project to minimize the need to precisely monitor these additional endpoints.

The oxygen analyzer used is a dual-channel unit that has two sample cells for measuring the partial pressure of oxygen in two airstreams simultaneously, expressed as percent oxygen (Applied Electrochemistry, Model S-3A, Sunnyvale, CA). Both cells are located in the same housing