HEMATOLOGIC AND IMMUNOLOGIC EFFECTS OF NONIONIZING ELECTROMAGNETIC RADIATION*

RALPH J. SMIALOWICZ, Ph.D.

Experimental Biology Division
Health Effects Research Laboratory
Environmental Protection Agency
Research Triangle Park, North Carolina

OVER the past several years much interest has been generated by reports of effects of nonionizing electromagnetic radiation (NEMR) on animal hematologic and immunologic systems. For the most part, these studies have been motivated by concern for the possible adverse health effects of NEMR for humans. Studies in which animals have been exposed at different frequencies and power intensities have shown inconsistent changes in elements of both systems. In some instances a thermal burden to the exposed animal has been credited with the observed changes, while in others a nonthermal or direct interaction of NEMR with the blood and blood-forming systems has been suggested to explain the observed effects. Traditionally, experiments performed in the Soviet Union and Eastern European countries have espoused the latter theory, an interpretation rooted in the approach that these investigators take in evaluating NEMR biological effects.

Historically, Soviet bloc countries have centered their research efforts on the effects of long-term exposure of animals and humans to low intensity fields. Consequently, many early reports on direct NEMR effects come from these countries. On the other hand, research in the West has, until more recently, been concerned with the potentially deleterious effects of NEMR fields of sufficient intensity to cause localized or generalized heat. In these studies, biological changes have been attributed to thermal stress. In any case, final interpretation of NEMR-induced changes requires consideration of many important factors that affect interaction of the NEMR field with the biological entity. Such variables as body shape,

*Presented as part of a *Symposium on Health Aspects of Nonionizing Radiation* sponsored by the Subcommittee on Public Health Aspects of Energy of the Committee on Public Health of the New York Academy of Medicine and held at the Academy April 9 and 10, 1979.

Address for reprint requests: MD-72, EPA, Environmental Research Center, Research Triangle Park, N.C. 27711.

mass, radiation frequency, duration of exposure, field intensity, specific absorption rate, energy distribution, orientation of body in the field, ambient environmental conditions, area of the body exposed, and field modulation may all influence final results.

This paper reviews the effects of NEMR on the hematologic and immunologic systems of humans and laboratory animals. Much of the Soviet literature is omitted, either because of lack of reported experimental detail or unavailability of English translations. For convenience, the review is divided into two general topics: clinical and epidemiological human studies and animal studies. The animal studies are divided into a discussion of hematologic and immunologic effects. The latter topic is subdivided into studies in which cellular components of the immune system have been exposed *in vitro* and studies dealing with whole body or *in vivo* exposures, including NEMR-induced hyperthermia.

Human Studies

Only a few clinical or epidemiological studies report the effects of NEMR on the hematological system of humans. Published reports on humans exposed to NEMR generally lack information regarding exposure history, and adequate control groups are often lacking or are nonrepresentative. Baranski and Czerski[1] discuss these and other problems inherent in such studies in their book.

Most clinical and epidemiological studies of NEMR come from the Soviet Union. Lysina[2] reported no significant difference in the circulating erythrocyte counts of 100 workers exposed to superhigh frequency (SHF) fields but gave no information about frequency, intensity, or duration of the exposure. He observed slight increases in reticulocyte counts in exposed personnel but no change in leukocyte counts. In another Soviet report, Sokolov et al.[3] examined 131 persons suffering various forms of radiowave sickness induced by exposure to SHF fields. They had received exposure to significant levels (several mW/cm.^{2}) in past years, but specifics about the exposure conditions, frequency, intensity, duration, etc. are not given. Sokolov et al.[3] reported a significant decrease in circulating thrombocytes and leukocytes due to neutropenia and relative lymphocytosis, a tendency toward reticulocytosis, increased bone-marrow erythronormoblasts, and an increase in the number of circulating cells undergoing mitosis. These hematologic effects, however, were reported as reversible, and cessation of exposure led to normal hemopoiesis in most

patients. These investigators[3] found no reason to believe that hypoplastic changes or leukemia follow exposure to SHF fields used in this study.

Few clinical or epidemiological studies in the United States have dealt with the possible health effects of NEMR. Daily[4] observed 45 men exposed to radar and high-frequency radiowaves for two months to nine years but gave no frequency or intensity levels. Periodic physical and blood examinations for 12 months revealed values within the normal range. Daily[4] reported "...no clinical evidence of damage to these personnel." Barron et al.[5] performed comprehensive physical examinations on radar personnel employed by an aircraft company. Two hundred twenty-six subjects with radar contact varying from occasional beam exposure to four hours a day and up to 13 years exposure were observed, although the frequency and intensity of the fields to which these individuals were exposed are not given. Barron et al.[5] mention that the radar bands most commonly associated with airborne equipment are the "S" and "X" bands near 2,900 MHz. and 9,000 MHz. respectively. Radar personnel were grouped by years of exposure and compared to controls of similar age. A significant decrease of polymorphonuclear cells was found in 25% of the radar personnel as compared to 12% in the control group. A marked increase in monocytes (above 6%) and eosinophiles (more than 4%) was detected in radar personnel, but the significance of these changes was not evaluated by these investigators.[5] Re-examination of 100 subjects after six to nine months of incidental contact with both "S" and "X" band radar revealed changes in erythrocyte counts, leukocyte counts, and relative numbers of polymorphonuclear cells. Barron et al.[5] found this "...paradoxical and difficult to interpret." In a later report, however, Barron and Baraff[6] stated that the changes were due to a variation in a laboratory technician's interpretation.

More recently, Lilienfeld et al.[7] evaluated the health of foreign service personnel stationed at the United States embassy in Moscow between 1953 and 1976. During and subsequent to this period of time, the American Embassy was irradiated with NEMR by the Soviets and exposure levels as high as 15 μW/cm.^{2} were recorded from June 1975 to February 1976. In this study the health of employees stationed in Moscow was compared with those stationed in other Eastern European posts during the same period. No differences between these groups in mortality or various morbidity measures were found. The authors concluded that "no convincing evidence was discovered that would directly implicate the exposure to

microwave radiation experienced by the employees at the Moscow embassy in the causation of any adverse health effects as of the time of this analysis.'' The authors noted several limitations in this study which influenced the probability of detecting statistically significant excess risks, problems in identification of the study population and classification of exposure status, incomplete response to health history questionnaires, and lack of adequate numbers.

Obviously, better defined clinical studies are needed to assess the human health effects of exposure to NEMR, perhaps by monitoring those occupationally exposed to NEMR.

Animal Studies

Hematology. Paucity of clinical and epidemiologic information on the health effects of NEMR has led to studies of the hematologic and immunologic systems of laboratory animals. Many early investigations of NEMR effects on the blood-forming system of laboratory animals employed field intensities of 10 mW/cm.^{2} and higher. For example, Diechmann et al.[8] reported significant leukocytosis, lymphocytosis, and neutrophilia in rats following seven hours of exposure to 24,000-MHz. pulse-modulated microwaves at 20 mW/cm.^{2} One week following exposure, peripheral blood values returned to normal. Rats exposed for three hours at 10 mW/cm.^{2} had the same changes, which returned to normal after two days. Increases in circulating erythrocytes, hemoglobin concentration, and hematocrit were observed in two strains of rats (Osborne-Mendel and CFN) exposed to 24,000 MHz. at 10 or 20 mW/cm.^{2}, but Fischer rats exposed under the same conditions had reduced circulating erythrocytes, hematocrit, and hemoglobin concentrations. No explanation for this discrepancy is given by the authors. In another experiment, Diechmann et. al.[9] exposed two dogs to 24,000-MHz. pulse-modulated (pulsed) fields at 24 mW/cm.^{2} One dog was exposed for 20 months, 6.7 hours per day, five days a week, while the second dog was exposed 16.5 hours per day four days a week. No significant changes were observed in blood volume, hematocrit, hemoglobin, erythrocytes, total and differential leukocytes, blood cholesterol, or protein-bound iodine. The only symptom attributed to the exposure was a slight loss in body weight. Two control dogs were employed in this study, but it was not indicated whether these dogs were sham or cage controls.

Kitsovskaya[10] exposed rats to 3,000-MHz. at 10, 40, or 100 mW/cm.^{2}

for various periods of time. No changes were found in rats exposed at 10 mW/cm.^{2} but at 40 and 100 mW/cm.^{2} circulating blood erythrocytes, leukocytes, and lymphocytes fell, and granulocytes increased in number. In contrast to the findings of Diechmann et al.,[8] these hematologic changes did not return to normal for months after cessation of exposures.

The apparent discrepancy between the results of Diechmann et al.[8] and Kitsovskaya[10] may be partially explained by the work of Michaelson et al.[11] These investigators reported that the hematopoietic effects of 2,800- and 1,280 MHz. pulsed fields depend upon the frequency, intensity, and duration of exposure. For example, dogs exposed to 2,800 MHz. had a marked decrease in circulating lymphocytes and eosinophils after six hours at 100 mW/cm.^{2} This exposure resulted in a mean rectal temperature increase of 1° C. Neutrophils remained slightly increased after 24 hours, while eosinophils and lymphocyte values returned to normal levels. After a two-hour exposure to 165 mW/cm.^{2} at 2,800 MHz., there was a slight leukopenia, neutropenia, and definite hemoconcentration. These changes were accompanied by a 1.7° C. rise in rectal temperature. Eosinopenia was still evident 24 hours after this exposure. General leukocytic changes were more apparent following exposure of dogs to 1,280 MHz. pulsed fields or to 200 MHz. continuous wave (CW) radiation. After exposure to 1,280 MHz. for six hours at 100 mW/cm.^{2}, dogs developed a leukocytosis and neutrophilia. After 24 hours the neutrophil level was still above pre-exposure levels. Both lymphocyte and eosinophil values were slightly depressed following exposure, but at 24 hours they were slightly higher than initial values. A six-hour exposure to 200 MHz. (CW) at 165 mW/cm.^{2} caused a marked increase in neutrophils and a slight decrease in lymphocytes. After 24 hours this trend was more evident. Michaelson et al.[11] concluded that the results indicated a stress response by the exposed animals brought about by stimulation of the hypothalmic or adrenal axis by the thermal influence of NEMR or both.

Spalding et al.[12] exposed mice to 800 MHz. fields at an average incident power density of 43 mW/cm.^{2} for two hours daily, five days a week for 35 weeks. These investigators found no changes in blood erythrocytes, leukocytes, hematocrit, or hemoglobin concentration, nor did the mean life span of control compared to exposed mice significantly differ. These investigators detected no changes in the peripheral blood picture of exposed mice, although a thermal burden was placed on these animals. Four mice died from thermal effects on the 33rd and 34th NEMR exposures.

In another long-term high incident power level study, Prausnitz and Susskind[13] exposed mice daily for 59 weeks to 9,270 MHz. pulsed microwaves at an average incident power density of 100 mW/cm.^{2} Mice were exposed for 4.5 minutes, after which an average body temperature rise of 3.5° C. was recorded. This regimen was calculated as one half the LD_{50} because nine minutes of exposure at this power density killed half of the mice. The longevity of the mice did not appear to be affected by this exposure according to these authors, but two pathological effects observed in this study were testicular degeneration and neoplasms of the white cells. One would expect testicular changes from the thermal load to the mice. Neoplasia was either monocytic or lymphatic leucosis or a lymphatic or myeloid leukemia. The authors make no attempt to explain this finding.

Effects at levels below 10 mW/cm.^{2} have also been reported. For example, Baranski[14,15] exposed guinea pigs and rabbits to continuous or pulse-modulated 3,000 MHz. microwaves at an average power density of 3.5 mW/cm.^{2} for three months, three hours daily. At this power level the body temperature of the animals was not elevated. Increases in absolute lymphocyte counts in peripheral blood, abnormalities in nuclear structure, and mitosis in the erythroblastic cell series in the bone marrow and in lymphoid cells in lymph nodes and spleen were observed. No changes were observed in peripheral blood granulocytes. Shifts in peripheral blood cells correlated with changes in the cellularity of the spleen and lymph nodes. An increase in the mitotic index and in the percentage of cells incorporating ^{3}H-thymidine was observed. Baranski[15] suggested that the specific effect causing the observed quantitative and qualitative differences in the white blood cell system were not a thermal effect of microwave radiation because experimental conditions excluded such a possibility. He stated that the mechanism should be investigated at the cellular level.

Djordjevic and Kolak[16] exposed rats to 2,400 MHz. (CW) at 10 mW/cm.^{2} for two hours daily for from 10 to 30 days. Body temperature in rats exposed under these conditions increased by 1° C. within the first 30 minutes of exposure, and remained at this level through the exposure period. Hematocrit, hemoglobin concentrations, and circulating erythrocytes in the exposed rats increased during the 30-day exposure, and fluctuations in the various leukocyte populations also fluctuated—changes that were thought to be due to the thermal effect of microwaves. In a more recent study, Djordjevic et al.[17] found no significant difference in any of several hematologic indices among rats which were exposed to 2,400

MHz. (CW) microwaves at 5 mW/cm.^{2} for one hour daily for 90 days.

Czerski[18] reported alterations in ferrokinetics in rabbits exposed to 2,950 MHz. continuous or pulse-modulated microwaves at 3 mW/cm.^{2} for two hours daily from 37 to 79 days. Erythrocyte production, measured by ^{59}Fe incorporation, significantly decreased in exposed animals but without change in erythrocyte count, hemoglobin level, or hematocrit. In another study, Czerski et al.[19] reported that the exposure of mice to 2,950 MHz. pulse-modulated microwaves at 1 mW/cm.^{2} at various times of the day caused changes in the normal circadian rhythm of bone marrow cell mitoses. Czerski et al.[19] suggested that microwaves may act as a mitotic stimulus for stem cells and particularly lymphocytes. The observed effects depended both on the time of the day when exposed and on the cell series. No differences were observed in rectal temperatures between the exposed mice and controls.

More recently, Rotkovska and Vacek[20] reported changes in hematopoietic cell populations of mice following a single five-minute exposure to 2,450 MHz. (CW) microwaves at an intensity of 100 mW/ cm.^{2} The response of microwave-exposed mice was compared to that of mice placed in a chamber at 43° C. for five minutes. Both treatments caused a rise in rectal temperature of over 2° C. Leukocytosis occurred both in mice exposed to microwaves and those exposed to heat but the time course for the leukocytosis differed: following microwave exposure, the total cell volume of the bone marrow and spleen decreased and the number of hematopoietic stem cells in bone marrow and spleen, as measured by the colony-forming unit assay, increased. Incorporation of ^{59}Fe in the spleen decreased 24 hours after microwave exposure, but the heat exposure decreased colony-forming units in bone marrow and spleen and increased the percentage of ^{59}Fe incorporation. Rotkovska and Vacek[20] concluded that the different effects on the hematopoietic stem cells of microwaves and externally applied heat suggests that biological effects caused by high intensities of NEMR are not necessarily related only to increased internal temperature. They indicated that their results suggest a possible direct effect. Their study is significant because it demonstrates a marked difference in the kinetic response of the hematopoietic system to two forms of heat stress. Consequently, these differences need to be considered in the interpretation of NEMR-induced changes in the hematopoietic system.

Rotkovska and Vacek[21] studied the effect of microwaves on the recovery of hematopoietic tissue following exposure to x-irradiation. Mice

exposed to whole-body x rays at 300 to 750 rads were subsequently exposed to 2,450 MHz. (CW) microwaves at different time intervals for five minutes at 100 mW/cm.^{2} The combined treatment accelerated the recovery of hematopoietic tissue, heightened erythropoiesis and myelopoiesis, and increased survival rate compared to x-irradiated mice. The increase in the number of hematopoietic endogenous colonies in the spleens of the x-irradiated mice followed microwave exposure supports Rotkovska and Vacek's[20] earlier observation of an elevation in the number of stem cells in the spleens of intact mice after microwave exposure alone. These investigators[21] suggested that microwaves may influence mechanisms that activate the stem-cell pool either by enhancing repair of sublethal radiation damage or by increasing proliferative capacity of stem cells that survive x-irradiation. This acceleration of the repair processes of radiation damage of hematopoietic cells following thermogenic doses of microwaves was thought to depend upon the stage of intracellular repair at the time of microwave exposure.[21] In earlier work, Michaelson et al.[22] reported that simultaneous exposure to x rays and microwaves (2,800 MHz., pulse-modulated, 100 mW/cm.^{2}) accelerated recovery of hematopoietic function in dogs. Exposure of x-irradiated (725 to 950 R) Chinese hamsters to microwaves (2,450 MHz. (CW), 60 mW/cm.^{2} for 30 minutes) five minutes following x-irradiation significantly increased the x ray $LD_{50(30)}$ compared to x rays alone or microwave exposure followed by x rays.[23] Lappenbush et al.[23] reported that the radioprotection of microwaves is associated with a delayed drop in the number of circulating white blood cells, reduced period of low cell density, and complete replenishment of white blood cells within 30 days following the dual treatment. Exposure to microwaves alone or in combination with x-ray exposure increased the relative number of neutrophils, reduced the relative number of lymphocytes, and slightly increased the number of circulating red blood cells. Animals exposed first to microwaves and then to x rays demonstrated more severe leukocyte changes than x-irradiated hamsters because leukocyte counts dropped faster and the animals developed leukopenia. These workers[23] suggested that the radioprotective effect of microwaves may be due to a thermal mechanism involving surviving bone marrow cells. These findings[20-23] indicate that NEMR at levels sufficiently intense to cause thermal loads in animals are capable of radioprotection against x rays. How this effect is accomplished is unknown.

The effect of NEMR whole body exposure on circulating blood cells of

developing rats has been studied in my laboratory.[24,25] Rats were exposed pre- and postnatally to 425-MHz. (CW) at 10 mW/cm.^{2} four hours daily for up to 40 to 41 days after birth. Because of growth of animals during this time, specific absorption rates (SARs) ranged from three to seven mW/g. Absolute neutropenia and relative lymphocytosis was observed in exposed compared to sham-control rats,[24] but these changes were not consistently reproduced. Rats exposed under the same regimen but to 2,450 MHz. (CW) at 5 mW/cm.^{2}, SAR = 1-5 mW/g., showed no difference in circulating erythrocyte count, total and differential leukocyte counts, hematocrit, and hemoglobin concentration when compared to sham-controls.[25] Hamrick and McRee[26] examined the effect of NEMR on developing birds. Quail eggs were exposed for 24 hours on the second day of incubation to 2,450 MHz. (CW) at 30 mW/cm.^{2}, SAR = 14 mW/g. At 24 to 36 hours after hatching, quails were examined for gross deformities, changes in organ weight, and hematological changes. No significant effects due to microwave exposure were detected.

In another study,[27] we exposed mice to 2,450 MHz. (CW) at 30 mW/cm.^{2}, SAR = 22 mW/g., for 30 minutes on 22 consecutive days. These mice showed no significant differences in circulating erythrocyte counts, total and differential leukocyte counts, hematocrit and hemoglobin concentration compared to sham-controls. Under the conditions of this study, microwave radiation did not elevate the rectal temperatures of exposed mice significantly more than among sham-controls. In contrast, when mice were exposed to thermogenic (2 to 4° C. rise in rectal temperature) levels of NEMR at 26-MHz. (CW), 8,610 mW/cm.^{2}, decreased numbers of circulating lymphocytes and increased circulating neutrophils were observed immediately following exposure.[28] Liburdy[28] reported that this shift reached its peak three hours after exposure. Pre-exposure levels of circulating lymphocytes and neutrophils returned to normal from 55 to 96 hours following exposure. On the other hand, mice exposed to high temperatures (79° C.) in a vented, dry-air oven showed an increase in circulating lymphocytes and neutrophils for 12 hours following exposure. It appears, therefore, that the response of circulating leukocytes to thermal loads depends on how heating of the body is accomplished. These results are similar to those reported by Rotkovska and Vacek,[21] and indicate that the heating properties of NEMR fields are unique compared to other modes of tissue heating.

In summary, thermogenic levels of NEMR elicit changes in the hematopoietic system that can for the most part be attributed to thermal stress response. Changes in the blood of animals exposed to NEMR at levels of intensity insufficient to increase body core temperature suggest a similar stress-response mechanism. Failure to record an increase in core temperature does not exclude the possibility that the animal can compensate for the added heat by thermoregulatory mechanisms. The response elicited by NEMR, however, seems to differ from that of conventional heating due to the unique heating property of this radiation. More sensitive methods to assess thermal stress responses are needed to explain observed hematologic phenomena more fully.

IMMUNOLOGY

In vivo studies. One of the most consistent findings of NEMR-induced changes in the hematopoietic system is increased lymphocyte formation and activity following exposure of several species to various frequencies of microwaves.[14,15,18,20]

Consequently, there have been several studies of the effects of NEMR on lymphocytes and the immune system. In a study by Czerski,[18] mice were exposed for six hours daily to 2,950 MHz. pulse-modulated microwaves at 0.5 mW/cm.^{2} for six or 12 weeks. After six weeks the relative number of lymphoblasts in the lymph nodes of exposed mice increased considerably. In another experiment,[18] rabbits were exposed two hours daily, six days weekly for six months to 2,950 MHz. pulsed microwaves at 5 mW/cm.^{2} Peripheral blood lymphocytes from these animals when cultured for seven days *in vitro* underwent increased "spontaneous lymphoblastoid transformation." Maximum increases occurred after one or two months of exposure, returned to base line, and rose again one month after irradiation had been terminated. Miro et al.[29] exposed mice to 3,105 MHz. pulsed microwaves continuously over a 145-hour period at an incident power of 2 mW/cm.^{2} Lymphoblastic cells in the spleen and lymphoid areas of exposed mice increased. A comparable response was observed[30] for lymphocytes cultured from Chinese hamsters exposed to 2,450 MHz. (CW) microwaves for 15 minutes on five consecutive days at 5 mW/cm.^{2}, SAR = 2.3 mW/g. Transformation to lymphoblastoid forms was maximum in cultures from hamsters exposed to 30 mW/cm.^{2}, SAR = 13.8 mW/g. This power density caused a 0.9° C. rise in rectal temperature

of exposed hamsters. Mitosis of lymphocytes cultured in the presence of the mitogen phytohemagglutinin (PHA) was depressed in cells obtained from hamsters exposed to 5, 15, 30, or 45 mW/cm.^{2} Cytogenic analysis of these lymphocytes revealed no difference in chromosomal aberrations between exposed and control hamsters. The significance of this study is that both enhancement of transformation and inhibition of mitosis were evident at 5 mW/cm.^{2}, a power density which caused no significant change in rectal temperature. These effects were transient and reversible with a return to control levels after 5 to 10 days.[30]

Prince et al.[31] reported a similar effect in rhesus monkeys, where they found an enhanced mitotic response of peripheral blood lymphocytes stimulated *in vitro* with PHA from monkeys three days following a 30-minute exposure to 10.5-MHz. pulsed radiation at 1,320 mW/cm.^{2} Enhancement of mitosis of cultured lymphocytes from monkeys similarly exposed to 19.27- and 26.6-MHz. were also reported, and increases in circulating lymphocytes from 4 to 47% above pre-exposure levels. At a frequency of 26.6 MHz., the rectal temperature of monkeys following exposure increased by 2.5° C. above pre-exposure levels. A frank thermal stress response at this frequency is the most plausible explanation for these results.

The particular susceptibilities of lymphocytes to NEMR described above have led to examination of the effects of nonionizing radiation on the immune system. For example, Czerski[18] reported that mice exposed for six weeks to 2,950 MHz. pulsed microwaves at 0.5 mW/cm.^{2} had significantly greater numbers of antibody-producing cells and higher serum antibody titers following immunization with sheep red blood cells (SRBC). Mice exposed for 12 weeks did not show this increased responsiveness.[18] More recently, Wiktor-Jedrzejczak et al.[32-34] exposed mice in a rectangular wave guide to 2,450 MHz. microwaves for 30 minutes at an average dose rate near 14 mW/g. At 3, 6, 9, and 12 days following single or multiple exposure, mice were tested for: the relative frequency of T (thymus-derived) and B (bone marrow-derived) splenic lymphocytes, the functional capacity of spleen cells to respond to T-and B-cell-specific mitogens, and ability to respond to SRBC or dinitrophenyllysine-Ficoll (DNP-lys-Ficoll). A single 30-minute exposure induced a significant increase in the proportion of complement-receptor positive lymphocytes (CR^+) in the spleens of mice which peaked six days following exposure. This effect was further enhanced by repeated (three times) exposures which also produced a

significant increase in the proportion of immunoglobulin positive (Ig^+) spleen cells.[33] A significant increase in the proportion of Fc receptor positive (FcR^+) cells in the spleens was observed seven days following single exposure for 30 minutes SAR = 13.7 mW/g. However, no change in the number of Ig^+ cells in spleens of these mice was observed.[34] The type and combination of surface receptors (CR, Ig, Fc) expressed on splenic B-cells represent different maturational stages in B-cell development. Wiktor-Jedrzejczak et al.[32-34] were unable to demonstrate any change in the total number of theta positive (θ^+) T-cells in the spleens of mice following a single or multiple exposure to 2,450 MHz. microwaves, nor change in *in vitro* spleen-cell response to stimulation by the T-cell-specific mitogens PHA and conconavalin A (Con A).[32] The response of spleen cells to pokeweed mitogen (PWM), which stimulates both T- and B-cells, was also unchanged. The response to the B-cell-specific mitogens lipopolysaccharide (LPS), polyinosinic polycytidylic acid (Poly I·C), and purified protein derivative of tuberculin (PPD), however, significantly increased over controls following a single exposure.[32] These results agree with the observed changes in the proportion of cells bearing different surface markers. Wiktor-Jedrzejczak et al.[32] noted that microwave irradiation did not stimulate lymphoid cell proliferation per se, but appeared to act as a polyclonal B-cell activator, which led to early maturation of noncommitted B-cells. These investigators also found a significant decrease in the primary immune response to SRBC, a thymus-dependent antigen, in mice immunized just prior to their first exposure to microwaves. They suggested that this decreased response may result from nonspecific microwave stimulation of some cells to mature before they are activated by antigen (SRBC), thereby increasing the proportion of unresponsive cells.[32]

We have[27] exposed mice to 2,450 MHz. (CW) under far field conditions for periods of 15 or 30 minutes daily for up to 22 consecutive days at power densities ranging from 5 to 35 mW/cm.^{2}, SAR = 4 to 25 mW/g. Splenic lymphocyte function was assessed by the *in vitro* mitogen-stimulated response assay as measured by ^{3}H-thymidine incorporation following culture in the presence of T-(PHA, Con A, PWM) or B-(LPS, PWM, PPD) mitogens. Frequencies of T (θ^+) and B (CRL^+) splenic cells and the primary immune response of mice to SRBC were also studied. No difference in the response to mitogens, SRBC, or in the frequency of T- or B-cells was observed in microwave-exposed compared to sham-exposed

mice. These experiments were performed in an attempt to reproduce, in part, the observations of Wiktor-Jedrzejczak et al.[32-34] Failure to repeat these observations was originally suggested as due to the different exposure systems,[27] but this discrepancy may also be due in part to the strain of mice used by these two different groups. Wiktor-Jedrzejczak et al.[32-34] used CBA/J mice while we[27] used BALB/C mice. Recent work (Dr. C. Schlagel, personal communication) has shown that the microwave-induced effect observed by the former group is strain specific in that it can be induced in CBA/J mice but not BALB/C mice. The reason for this strain specificity is unexplainable at this time.

Liburdy[35] recently reported that changes in splenic lymphocyte populations similar to those observed by Witkor-Jedrzejczak et al.[32-24] can be produced by exposure of mice to thermogenic levels of 26 MHz. radiofrequency radiation. When mice were exposed to 26 MHz. at an intensity which produced a 2 to 3° C. rise in rectal temperature, splenic T- and B-lymphocytes relatively increased. Similar responses were induced following administration of methyl prednisolone sodium succinate, results that suggest that these radiofrequency-induced changes represent a stress phenomenon.

Microwave effects on the development of the immune response have been studied in two laboratories. We[24,25] exposed rats starting on day six of gestation through 41 days from birth to 2,450 MHz. (CW) at 5 mW/cm.^{2}, SAR = 1 to 5 mW/g. Their lymphocytes responded to a significantly greater extent than those from control animals following stimulation *in vitro* with T- or B-cell mitogens.[25] A similar increase in lymphocyte responsiveness was seen in lymphocytes from rats exposed to 425 MHz. (CW).[24] In this study, rats were exposed pre- and postnatally to 425 MHz. microwaves at 10 mW/cm.^{2}, SAR = 3 to 7 mW/g., for up to 41 days following birth. These two studies[24,25] suggest that long-term exposure of developing rats to microwaves may cause the increased responsiveness of cultured lymphocytes. These results resemble other reported changes in lymphocyte responsiveness following NEMR exposure.[18,30-34] Hamrick et al.[36] examined the humoral immune response of Japanese quails exposed to microwaves during embryogenesis. Fertile quail eggs were exposed to 2,450 MHz. (CW) microwaves at 5 mW/cm.^{2}, SAR = 4.03 mW/g., throughout the first 12 days of development. At five weeks of age quails were immunized with SRBC and the levels of anti-SRBC antibodies were determined. No difference was observed in antibody titers of exposed and

sham-exposed quails. Further, microwave exposure did not significantly alter the weights of the bursa of Fabricius (site of B-cell production in birds) and spleen.

NEMR-induced effects on the phagocytic leukocytes of animals have been reported by Szmigielski et al.[37] Rabbits were exposed to 3,000 MHz. for six hours daily for six weeks to three months at 3 mW/cm.^{2} After the last exposure to microwaves, rabbits were infected with an intravenous injection of virulent *Staphylococcus aureus*. At periods before and after infection, functional tests of granulopoiesis documented decreased production of mature granulocytes in response to infection in microwave exposed rabbits which were more seriously ill than controls.

Exposure of laboratory animals to NEMR can change the functional integrity of lymphocytes that are important in the immune defense system of man and animals. The significance of the changes caused by NEMR is difficult to interpret. While some studies indicate that NEMR increases responsiveness of lymphocytes[18,24,25,31-35] and potentiates the immune response to antigen,[18] others indicate depressed responsiveness.[30,32,35,37] In most cases these alterations can be attributed to stress-type responses because similar changes are observed at thermogenic levels of NEMR[30,31,35] or following administration of glucocorticoids.[35] Effects observed at lower levels of NEMR also appear to involve some type of stress-response mechanism. Fully to evaluate these low-level effects, a better understanding of the interaction of the immune and thermoregulatory systems is needed.

In vitro studies. Several studies have attempted to determine whether *in vitro* exposure of lymphocytes with NEMR leads to "direct" changes in their metabolic or functional states. In an early study, Stodolnik-Baranska[38] exposed human lymphocytes in culture to 3,000 MHz. pulsed microwaves at 7 or 14 mW/cm.^{2} Lymphocytes were irradiated for four hours daily at 7 mW/cm.^{2} for three to five days, while those exposed to 14 mW/cm.^{2} were irradiated for 15 minutes for three to five days. After five days in culture, the microwave-exposed cells had undergone a fivefold increase in blast transformation compared to controls. Czerski[18] attempted to repeat this experiment but found the results poorly reproducible. In a more recent study, Baranski and Czerski[39] reported that exposure of human lymphocytes to 10,000 MHz. at power densities between 5 and 15 mW/cm.^{2} could induce lymphoblastoid transformation. At power densities below 5 mW/cm.^{2} this effect was not observed, while at power levels

above 20 mW/cm.^{2} cell viability decreased. The induction of blast transformation depended upon stopping the exposure (5 to 15 mW/cm.^{2}) at the moment when the temperature of the medium reached 38° C. These results suggest that the microwave-induced blast transformation is due to a thermal effect.

Similar increases in the lymphoproliferative response of cells exposed to temperatures greater than 37° C. have been reported. Ashman and Nahmais[40] reported that human lymphocytes, when cultured at 39° C. with the mitogens PHA or Con A, showed an enhancement and earlier onset of ^{3}H-thymidine incorporation compared with cultures incubated at 37° C. In a similar study, Roberts and Steigbigel[41] reported that the *in vitro* human lymphocyte response to PHA and the common antigen streptokinase-streptodornase was enhanced at 38.5° C. relative to 37° C. Smith et al.[42] reported that the *in vitro* response of human lymphocytes to PHA, Con A, PWM, and allogeneic lymphocytes in mixed lymphocyte (MLS) was markedly enhanced by culture at 40° C. compared to 37° C. These studies demonstrate the need to monitor and to control the temperature of cultures exposed to NEMR. Without adequate temperature data, it is virtually impossible to accept *in vitro* effects as due to NEMR itself.

Failure to increase culture temperature during or following *in vitro* NEMR exposure has been shown in several studies not to affect the proliferative response of lymphocytes. Holm and Schneider[43] exposed human lymphocytes, cultured in the presence of PHA, to 27.12-MHz. at an estimated effective radiating power of 10 W. No substantial differences were noted between 27.12 MHz. exposed cultures and controls regarding DNA synthetic index, growth, or mitotic index. Culture temperatures did not exceed those of controls (37° C.) by more than 1° C. In my laboratory[44] murine splenic lymphocytes were exposed to 2,450 MHz. (CW) microwaves for one, two, or four hours at 10 mW/cm.^{2}, SAR near 19 mW/g. Following irradiation, the temperature of the exposed cultures did not differ significantly from controls and cell viability was unchanged. Following irradiation, cells were cultured for 72 hours in the presence of T- or B-cell mitogens and the proliferative response was measured by ^{3}H-thymidine incorporation. No difference was found in the blastogenic response of microwave-exposed and sham-exposed spleen cells to any of the mitogens employed. In a similar experiment, Hamrick and Fox[45] exposed rat lymphocytes to 2,450 MHz. (CW) microwaves for four, 24, or 44 hours at 5, 10, or 20 mW/cm.^{2}, SAR = 0.7, 1.4 and 2.8 mW/g.

respectively. Unlike the previous studies,[18,38,43,44] Hamrick and Fox[45] exposed whole blood preparations to microwaves. Transformation of unstimulated or PHA-stimulated lymphocytes was measured using ^{3}H-thymidine. No significant differences were found in the proliferative capacity of lymphocytes from exposed and control cultures. The effects of 2,450 MHz. CW microwave radiation on the growth and viability of cultured human lymphoblasts was studied by Lin and Peterson.[46] Human lymphoblasts (lines Daudi and HSB_2) were exposed to 2,450 MHz. (CW) microwaves in a waveguide for 15 minutes at incident power densities of 10 to 500 mW/cm.^{2} The corresponding rates of energy absorption were up to 1,200 mW/g. No temperature increase was found, even at the highest power density in the capillary tube which held the cell suspension in the waveguide. No change was observed in the viability or growth of microwave-exposed lymphoblasts compared to controls—further evidence that in the absence of heating, no change in lymphocyte activity occurs following NEMR exposure *in vitro*.

In vitro exposure of macrophages to 2,450 MHz. has been reported to depress phagocytosis by Mayers and Habeshaw.[47] Monolayer cultures of mouse peritoneal macrophages were perfused with suspensions of human erythrocytes while simultaneously exposed to 2,450 MHz. microwaves at 50 mW/cm.^{2} The energy absorbed as heat by the sample was 15 J/min. The phagocytic index of exposed cultures was significantly lower than control after a 30-minute exposure. Macrophage phagocytic activity was restored to normal if the microwave irradiation was discontinued. When the microwaves were on, a 2.5° C. temperature increase was observed, but the final temperature in any given experiment did not exceed 36.2° C. These investigators concluded that the observed depression of phagocytosis in irradiated cultures was not thermally induced and that the 2.5° C. rise in temperature during irradiation would have been expected to enhance rather than depress phagocytosis because optimal phagocytosis occurs at a temperature of 38.5° C.[47] The mechanism by which this effect is caused is not known, but heating effects are difficult to dismiss at such a high power density. While the temperature of the suspension medium did not exceed 36.2° C., thermal gradients of much higher temperature would be expected at the macrophage-glass interface.

A microwave-induced effect on granulocyte integrity and viability was reported by Szmigielski,[48] who exposed rabbit granulocytes *in vitro* to 3,000 MHz. (CW) microwaves at 1 or 5 mW/cm.^{2} for 15, 30, or 60

minutes. Cultures exposed at 5 mW/cm.^{2} for 30 or 60 minutes had increased cell death as demonstrated by an increase in nigrosine staining and enhanced liberation of lysosomal enzymes. Exposure to 1 mW/cm.^{2} fields did not cause increased cell death but led to a partial liberation of hydrolases. No change was observed in the temperature of microwave-exposed cultures. The liberation of granulocyte acid phosphatase and lysozyme was observed in cell suspensions exposed to either 1 or 5 mW/cm.^{2} and both exhibited a time- and dose-dependent relation. Szmigielski[48] suggested that low-level microwaves may affect the cellular membrane. The possible production of thermal gradients produced in the culture vessels by NEMR might explain these effects.

NEMR-induced hyperthermia studies. Over the past several decades increasing evidence for the beneficial effects of partial or whole-body hyperthermia has accumulated. Several studies have demonstrated that NEMR-induced hyperthermia may benefit a variety of diseases, including cancer. For example, LeVeen et al.[49] reported that radiofrequency therapy (13.56 MHz.) at power densities of 1,000 to 4,000 mW/cm.^{2} for up to 30 minutes at a time produced tissue necrosis or substantial regression of cancer in 21 patients. Combined radiotherapy (x rays) and NEMR-induced hyperthermia (434 MHz.) is reported to cause a 94% resolution of primary and secondary lesions and to increase the three-year survival rate of patients with advanced head and neck cancers.[50]

While in most cases destruction of heated tissue is the ultimate goal, such applications of NEMR have often led to changes in the immune response. For example, Shah and Dickson[51] reported that following local heating of VX2 (carcinoma) tumor-bearing rabbits with a radiofrequency generator (13.56-MHz.), tumor regression and host cure were observed in 70% of the rabbits. Intratumor temperatures of 47-50° C. were achieved within 30 minutes. Along with tumor regression, cell-mediated immunity, as measured by skin reactivity to tumor extract and dinitrochlorobenzene, markedly increased. A 100-fold increase in serum levels of antitumor antibody and increased response to antigen bovine serum albumin were also observed. Total-body hyperthermia, however, led to temporary restraint of tumor growth, followed by a return to an exponential increase in tumor volume and rapid death of the rabbit. This course of events following whole-body hyperthermia was accompanied by abrogation of the enhanced cellular and humoral immune responsiveness observed following radiofrequency-induced local heating.

Szmigielski et al.[52] reported that local heating (43° C.) of the Guérin epithelioma in Wistar rats by 2,450 MHz. (CW) microwaves both inhibited tumors and stimulated the immune reaction against the tumor. Nonspecific immune reactions stimulated by this treatment were the antibody response to BSA, high reactivity of spleen lymphocytes to the mitogen PHA, and increased serum lysozyme levels as a measure of macrophage activity. Tumor-specific reactions observed were increased cytotoxicity of spleen cells and peritoneal macrophages to cultured tumor cells. Similar results were reported by Marmor et al.,[53] who exposed tumors in mice to local 1,356 MHz. radiation. EMT-6 tumors were found to be highly sensitive to cure by radiofrequency heating. The cure rate was a function of temperature and duration of exposure: a five-minute exposure at 44° C. cured almost 50% of the tumors. To determine the effectiveness of radiofrequency heating on tumor regression, tumor-cell-survival studies were done on EMT-6 tumors treated in situ. Cell inactivation by radiofrequency heating was similar to that for hot water bath heating. The results indicated that direct cell killing could not account for the observed cures, and these investigators[53] suggested that hyperthermia may stimulate a tumor-directed immune response.

Szmigielski et al.[54] exposed mice bearing transplanted sarcoma-180 tumors for two hours daily on the first through 14th day after transplantation to 3,000 MHz. microwaves at 40 mW/cm.^{2} This exposure led to a 3° to 4° C. increase in rectal temperature, and resulted in a reduction of tumor mass by approximately 40%, a reduction enhanced when microwave hyperthermia was combined with Colcemide, Streptolysin S, or both. Colcemide enhances the inhibiting effect of microwaves on proliferation of cells *in vitro*[55] and Streptolysin S is an antineoplastic substance. Szmigielski et al.[52] suggested that immunostimulation is important in the complex inhibition of tumor growth by increased temperatures.

While many have heralded local and systemic hyperthermia as a possible cancer treatment, either alone or in combination with drugs or ionizing radiation, there is evidence that hyperthermia may enhance the dissemination of certain cancers and abrogate the immune response. For example, Dickson and Ellis[56] reported that local hyperthermia (hot water bath immersion) of implanted solid Yoshida sarcomas in the feet of rats can enhance dissemination of this sarcoma if local heating is inadequate for complete tumor destruction. Walker et al.[57] reported a dramatic promotion of metastasis by local heating (water heater) of the C3H mouse mammary

carcinoma. Shah and Dickson[58] exposed normal rabbits to either radiofrequency-induced (13.56 MHz.) or watercuff local hyperthermia of thigh muscles which were maintained at 42° C. for one hour on three consecutive days. No alteration in the response to dinitrochlorobenzene challenge was observed. However, the humoral immune response to bovine serum albumin was significantly depressed. This response was independent of the method and degree of heating. The results suggest that B-lymphocytes are more susceptible to hyperthermic damage than are T-lymphocytes.

Discrepancies in the response of various species with different neoplasms to NEMR-induced or heat-induced hyperthermia indicates that more work in this area is needed. It is not known whether NEMR-induced hyperthermia affords the host more immunologic benefit than conventional heating of tissue. What is certain, however, is that NEMR-generating devices may contribute to cancer treatment to providing a means to generate intense heat in a localized defined area of tissue.

Discussion

It is apparent that partial or whole-body exposure of animals to NEMR may lead to a variety of changes in their hematologic and immunologic systems. These changes are often transient, with blood counts or other responses returning to normal either immediately or soon after cessation of exposure. However, inconsistencies in these responses to NEMR adds to the ambiguity of the results. In most reports, the thermal influence of NEMR on the observed alterations is obvious, and increased body core temperature and hematologic and immunologic changes are directly correlated in several reports. Observed results are explainable in terms of NEMR-induced thermal stress. Several reported effects of NEMR on the hematologic and immune systems, for example, are very similar to those following a stress response involving the hypothalamic-hypophyseal-adrenal axis or following administration of glucocorticoids.[59-67] Other observed changes, however, are more difficult to explain on a thermal-stress basis, primarily because of a lack of sensitive techniques to detect subtle stress responses. Mere lack of a rectal temperature increase following exposure to NEMR, however, does not exclude a possible thermal interaction which the animal can compensate and control. Localized heating or "hot spots" in organs critical to the hematopoeitic and immune systems may occur from production of thermal gradients unique to radiofrequency

energy absorption by biological systems. Interpretation of NEMR effects on the blood and blood-forming systems depends to a great degree on the absorption characteristics of biological materials and the thermoregulatory system of the irradiated individual. Many factors affect the dose rate of NEMR to the body, organ, or tissue, including the frequency and wave length, field intensity, direction of wave propagation, the mass and shape of the body, and orientation of the body in the NEMR field. The rate at which energy is absorbed per unit of body mass is called the specific absorption rate (SAR) or the dose rate. The rate of whole body energy deposition varies with frequency and is greatest at the resonant absorption frequency. In addition to other factors, the resonant frequency for an individual object depends on its size and mass. For example, an adult human in free space will maximally absorb NEMR at a frequency between 70 and 80 MHz., while the resonant frequency for a mouse is about 2,450 MHz.[68] Unfortunately, few of the reports cited above[24-28,30,32-36,44-46] give the SAR measurements, which are extremely important in the evaluation of biological effects. Because of discontinuities in the dielectric properties of tissues, standing waves with the concomitant production of localized heating or "hot spots" are produced by NEMR fields. Both the frequency and the conductivity and permittivity of irradiated tissue affect the propagation and absorption of NEMR in tissue. For example, blood, body fluids, skin, muscle, brain and internal organs that contain large amounts of water will absorb more NEMR than such tissue with low water content as bone, fat, and tendon.[69] Consequently, actual distribution of energy within a body and the specific absorption rate must be determined before one can legitimately ascribe an observed change as due to "direct" interaction of NEMR fields in the absence of some form of thermal involvement.

The interpretation of NEMR-induced changes in the hematologic or immunologic systems also depends upon the ambient exposure conditions and thermoregulatory capacity of the exposed individual. The dissipation of NEMR-induced thermal loading among species depends upon the area of the body exposed, duration of exposure, efficiency of heat elimination which includes thermoregulation and blood flow, and such ambient environmental conditions as temperature, humidity, and air flow. Humans are relatively good at thermoregulation, whereas such small rodents as mice are poor thermoregulators. This is an important consideration in attempting to extrapolate changes observed in rodents following NEMR exposure and

what might be expected to occur in humans. Consideration of frequency scaling and the equal whole-body SAR concept are essential to attempt extrapolation of NEMR effects between animals and humans. At best, this is only a gross approximation, because sizes and shapes affect the distribution of NEMR energy within bodies. Therefore, comparisons of the distribution of energy in an animal to that of a man is very difficult and consequently extrapolation of NEMR-induced effects becomes even more difficult.

Unfortunately, the few available clinical and epidemiological studies are of limited usefulness in our attempt to evaluate the health effects of NEMR. For the most part, the studies cited above are inadequate to assess effects on the hematologic and immunologic systems of humans exposed to NEMR. These studies lack sufficient detail of exposure history and usually lack adequate control groups. While none of the studies report detrimental hematologic changes, one wonders why more and better conducted studies have not been done. Several occupational groups would be very well suited for ongoing clinical studies in that NEMR exposure records could be kept and adequate controls employed. Three prime candidates for such studies would be radiofrequency tower maintenance personnel, personnel who operate radiofrequency sealers, and diathermy unit operators. However, without further clinical studies, it is difficult to make a definitive statement on the human health effects of NEMR.

In conclusion, high intensity NEMR fields induce thermal loads in animals which in turn affect the hematologic and immunologic systems. These responses are similar if not identical to responses elicited in animals following a stressful encounter or administration of glucocorticoids. More subtle NEMR-induced heating may account for the biological effects reported in the absence of an increase in body-core temperature. These effects, similar to stress-type responses, may be attributed to the unique heating property of NEMR. It is not certainly known that these effects are necessarily the hallmark of changes in the hematologic or immunologic systems that will eventually lead to disease or, for that matter, to a more responsive immune system.

Convincing evidence for a direct interaction of NEMR with hematopoietic cells *in vitro* or *in vivo* is not available. Evidence is increasing that NEMR fields may interact and cause alterations at the membrane level of organization in nervous tissue,[70,71] but no such clear evidence is available for blood cells. This does not exclude the possibility of such

interactions, and continued research in this area of mechanism of action is necessary. What appears to be evident is that the hematological and immunologic systems are sensitive to NEMR fields. The relative sensitivities of these systems for NEMR fields needs to be determined so that the potential health risk to humans can be better evaluated. Because of lack of understanding of the effects of long-term, low-level exposure to NEMR on the hematologic and immunologic systems of man and animals, future studies are needed. Through such studies it is hoped that a reasonable understanding of NEMR effects can be achieved so that unfounded restrictions on the beneficial uses of nonionizing radiation are avoided.

DISCLAIMER

This report has been reviewed by the Office of Research and Development, EPA, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

REFERENCES

1. Baranski, S. and Czerski, P.: Health Status of Personnel Occupationally Exposed to Microwaves. Symptoms of Microwave Overexposure. In: *Biological Effects of Microwaves*. Stroudsburg, Pa., Dowden, Hutchinson and Ross, 1976, pp. 153-69.
2. Lysina, G. G.: Effect of superhigh frequencies on the morphological composition of blood. *Gigi. Sanit. 30:*95-96, 1965.
3. Sokolov, V. V., Gribova, I. A., Chulina, N. A., et al.: State of the Blood System Under the Influence of SHF Fields of Various Intensities and in Radiowave Sickness. In: *Biological Effects of Radiofrequency Electromagnetic Fields*, Gordon, Z. V., editor. Springfield, Va., Nat. Tech. Information Service, 1974, pp. 63-71.
4. Daily, L. E.: A clinical study of the results of exposure of laboratory personnel to radar and high frequency radio. *U.S. Navy Med. Bull.* 41:1052-56, 1943.
5. Barron, C. I., Love, A. A., and Baraff, A. A.: Physical evaluation of personnel exposed to microwave emanations. *J. Aviat. Med. 26:*442-52, 1955.
6. Barron, C. I. and Baraff, A. A.: Medical considerations of exposure to microwaves (radar). *J. A. M. A. 168:*1194, 1958.
7. Lilienfeld, A. N., Tonascia, J., Tonascia, S., et al.: Summary and Conclusion. In: *Foreign Service Health Status Study*. Baltimore, Johns Hopkins University School of Hygiene and Public Health, 1978.
8. Deichmann, W. B., Miale, J., and Landeen, K.: Effect of microwave radiation on the hemopoietic system of the rat. *Toxicol. Appl. Pharmacol. 6:*71-77, 1964.
9. Deichmann, W. B., Beinal, E., Stephens, F., and Landeen, K.: Effects on dogs of chronic exposure to microwave radiation. *J. Occup. Med. 5:*418-25, 1963.
10. Kitsovskaya, I. A.: The effect of centi-

meter waves of different intensities on the blood and hemopoietic organs of white rats. *Gig. Tr. Prof. Zabol. 8:*14, 1964.
11. Michaelson, S. M., Thompson, R. A. E., El Tamami, M. Y., et al.: The hematologic effects of microwave exposure. *Aerospace Med. 35:*824-29, 1964.
12. Spalding, J. F., Freyman, R. W., and Holland, L. M.: Effects of 800-MHz electromagnetic radiation on body weight, activity, hematopoiesis and life span in mice. *Health Phys. 20:*421-24, 1971.
13. Prausnitz, S. and Susskind, C.: Effects of chronic microwave irradiation on mice. *IRE Trans. Biomed. Electron. 9:*104-08, 1962.
14. Baranski, S.: Effect of chronic microwave irradiation on the blood forming system of guinea pigs and rabbits. *Aerospace Med. 42:*1196-99, 1971.
15. Baranski, S.: Effect of microwaves on the reactions of the white blood cells system. *Acta Physiol. Polonica 23:*685-95, 1972.
16. Djordjevic, Z. and Kolak, A.: Changes in the peripheral blood of the rat exposed to microwave radiation (2400-MHz) in conditions of chronic exposure. *Aerospace Med. 44:*1051-54, 1973.
17. Djordjevic, Z., Lazarevic, N., and Djokoic, V.: Studies on the hematologic effects of long-term, low-dose microwave exposure. *Aviat. Space Environ. Med. 48:*516-18, 1977.
18. Czerski, P.: Microwave effects on the bloodforming system with particular reference to the lymphocyte. *Ann. N.Y. Acad. Sci. 247:*232-42, 1975.
19. Czerski, P., Paprocka-Slonka, E., and Stolarska, A.: Microwave irradiation and the circadian rhythm of bone marrow cell mitosis. *J. Microwave Power 9:*31-37, 1974.
20. Rotkovska, D. and Vacek, A.: The effect of electromagnetic radiation on the hematopoietic stem cells of mice. *Ann. N.Y. Acad. Sci. 247:*243-50, 1975.
21. Rotkovska, D. and Vacek, A.: Modification of repair of X-irradiation damage of hemopoietic system of mice by microwaves. *J. Microwave Power 12:* 119-23, 1977.
22. Michaelson, S. M., Thomson, R. A. E., Odland, L. T., and Howland, J. W.: The influence of microwaves or ionizing radiation exposure. *Aerospace Med. 34:*111, 1963.
23. Lappenbush, W. L., Gillespie, L. J., Leach, W. M., and Anderson, G. E.: Effect of 2450-MHz microwaves on the radiation response of X-irradiated Chinese hamsters. *Rad. Res. 54:*294-303, 1973.
24. Smialowicz, R. J., Weil, C. M., Kinn, J. B., and Elder, J. A.: Exposure of rats to 425-MHz CW microwave radiation: Effects on lymphocytes. *J. Microwave Power.* In press.
25. Smialowicz, R. J., Kinn, J. B., and Elder, J. A.: Perinatal exposure of rats to 2450-MHz CW microwave radiation: Effects on lymphocytes. *Radio Sci.* (Suppl.) *14:*1979. In press.
26. Hamrick, P. E. and McRee, D. I.: Exposure of the Japanese quail embryo to 2.45-GHz microwave radiation during the second day of development. *J. Microwave Power 10:*211-21, 1975.
27. Smialowicz, R. J., Riddle, M. M., Brugnolotti, P. L., et al.: Evaluation of lymphocyte function in mice exposed to 2450-MHz CW microwaves. In: *Proc. 1978 Symp. Electromagnetic Fields in Biological Systems,* Stuckly, S. S., editor. Edmonton, Canada, Int. Microwave Power Inst., 1979, pp. 122-52.
28. Liburdy, R. P.: Effects of radiofrequency radiation on inflammation. *Radio Sci.* (Suppl.) *12:*179-83, 1977.
29. Miro, L., Toubiere, R., and Pfirster, A.: Effects of Microwaves on the Cell Metabolism of the Reticulohistocytic System. In: *Biological Effects and Health Hazards of Microwave Radiation* (proceedings of an international symposium). Czerski, P., Ostrowski, K., Shore, M. L., et al., editors. Warsaw, Poland, Polish Med. Pub., 1974, pp. 89-97.
30. Huang, A. T., Engel, M. E., Elder, J. A., et al.: The effect of microwave radi-

ation (2450-MHz) on the morphology and chromosomes of lymphocytes. *Radio Sci.* (Suppl.) *12:*173-77, 1977.

31. Prince, J. E., Mori, L. H., Frazer, J. W., and Mitchell, J. C.: Cytologic aspect of radiofrequency radiation in the monkey. *Aerospace Med. 43:*759-61, 1972.
32. Wiktor-Jedrzejczak, W., Ahmed, A., Czerski, P., et al.: Immune response of mice to 2450-MHz microwave radiation: Overview of immunology and empirical studies of lymphoid splenic cells. *Radio Sci.* (Suppl.) *12:*209-19, 1977.
33. Wiktor-Jedrzejczak, W., Ahmed, A., Sell, K. W., et al.: Microwaves induce an increase in the frequency of complement receptor-bearing lymphoid spleen cells in mice. *J. Immunol. 118:*1499-1502, 1977.
34. Wiktor-Jedrzejczak, W., Ahmed, A., Czerski, P., et al.: Increase in the frequency of FC receptor (FCR) bearing cells in the mouse spleen following a single exposure of mice to 2450-MHz microwaves. *Biomedicine 27:*250-52, 1977.
35. Liburdy, R. P.: Radiofrequency radiation alters the immune system: Modulation of T- and B-lymphocyte levels and cell-mediated immunocompetence. *Rad. Res. 77:*34-46, 1979.
36. Hamrick, P. E., McRee, D. I., Thaxton, P., and Parkhurst, C. R.: Humoral immunity of Japanese quail subjected to microwave radiation during embryogeny. *Health Phys. 33:*23-33, 1977.
37. Szmigielski, S., Jeljaszewicz, J., and Wiranowska, M.: Acute staphylococcal infections in rabbits irradiated with 3-GHz microwaves. *Ann. N.Y. Acad. Sci. 247:*305-11, 1975.
38. Stodolnik-Baranska, W.: Lymphoblastoid transformation of lymphocytes *in vitro* after microwave irradiation. *Nature 214:*102-03, 1967.
39. Baranski, S. and Czerski, P.: Biological Effects of Microwaves. Experimental Data. In: *Biological Effects of Microwaves.* Stroudsburg, Pa., Dowden, Hutchinson and Ross, 1976, p. 133.
40. Ashman, R. B. and Nahmias, A. J.: Effect of incubation temperature on mitogen responses of lymphocytes from adult peripheral blood and from cord blood. *Clin. Exp. Immunol. 33:*319-26, 1978.
41. Roberts, N. J. and Steigbigel, R. T.: Hyperthermia and human leukocyte functions: Effects on response of lymphocytes to mitogen and antigen and bactericidal capacity of monocytes and neutrophils. *Infect. Immun. 18:*673-79, 1977.
42. Smith, J. B., Knowlton, R. P., and Agarwal, S. S.: Human lymphocyte responses are enhanced by culture at 40°C. *J. Immunol. 121:*691-94, 1978.
43. Holm, D. A. and Schneider, L. K.: The effects of nonthermal radio frequency radiation on human lymphocytes *in vitro*. *Experientia 26:*992-94, 1970.
44. Smialowicz, R. J.: The Effect of Microwaves (2450-MHz) on Lymphocyte Blast Transformation *In Vitro*. In: *Biological Effects of Electromagnetic Waves, Selected Papers of the USNC/URSI Annual Meeting,* Boulder, Co., October 20-23, 1975, Johnson, C. C. and Shore, M. L., editors. HEW Publication No. 77-8010. Washington, D.C., Govt. Print. Off., 1976, vol. 1, pp. 472-83.
45. Hamrick, P. E. and Fox, S. S.: Rat lymphocytes in cell culture exposed to 2450-MHz (CW) microwave radiation. *J. Microwave Power 12:*125-32, 1977.
46. Lin, J. C. and Peterson, W. D.: Cytological effects of 2450-MHz CW microwave radiation. *J. Bioeng. 1:*471-78, 1977.
47. Mayers, C. P. and Habershaw, J. A.: Depression of phagocytosis: A nonthermal effect of microwave radiation as a potential hazard to health. *Int. J. Radiat. Biol. 24:*449-61, 1973.
48. Szmigielski, S.: Effect of 10-cm (3-GHz) electromagnetic radiation (microwaves) on granulocytes *in vitro*. *Ann. N.Y. Acad. Sci. 247:*275-80, 1975.
49. LeVeen, H. H., Wapnick, S., Piccone, V., et al.: Tumor eradication by radiofrequency therapy. Response in 21 patients. *J. A. M. A. 235:*2198-2200,

1976.
50. Nelson, A. J. M. and Holt, J. A. G.: Combined microwave therapy. *Med. J. Aust. 2:*88-90, 1978.
51. Shah, S. A. and Dickson, J. A.: Effect of hyperthermia on the immunocompetence of VX2 tumor-bearing rabbits. *Cancer Res. 38:*3523-31, 1978.
52. Szmigielski, S., Janiak, M., Hryniewicz, W., et al.: Local microwave hyperthermia (43°C) and stimulation of the macrophage and T-lymphocyte systems in treatment of Guérin epithelioma in rats. *Z. Krebsforsch. 91:*35-48, 1978.
53. Marmor, J. B., Hahn, N., and Hahn, G. M.: Tumor cure and cell survival after localized radiofrequency heating. *Cancer Res. 37:*879-83, 1977.
54. Szmigielski, S., Pulverer, G., Hryniewicz, and Janiak, M.: Inhibition of tumor growth in mice by microwave hyperthermia, streptolysin S and colcemide. *Radio Sci.* (Suppl.) *12:*185-89, 1977.
55. Szmigielski, S., Kobus, M., and Janiak, M.: Enhanced cytotoxic effect of hyperthermia (43°C) on colcemide-treated normal and SV_{40}-transformed cells grown *in vitro*. *Z. Geschwalstkrankh. 47:*396-99, 1976.
56. Dickson, J. A. and Ellis, H. A.: The influence of tumor volume and the degree of heating on the response of the solid Yoshida sarcoma to hyperthermia (40-42°C). *Cancer Res. 36:*1188-95, 1976.
57. Walker, A., McCallum, H. M., Wheldon, T. E., Nias, A. H. W., and Abdelaal, A. S.: Promotion of metastasis of C3H mouse mammary carcinoma by local hyperthermia. *Br. J. Cancer 38:*561-63, 1978.
58. Shah, S. A. and Dickson, J. A.: Effect of hyperthermia on the immune response of normal rabbits. *Cancer Res. 38:* 3518-22, 1978.
59. Zimin, Y. I.: An increased number of hematopoietic stem cells in mice in the initial period of the stress reaction. *Bull. Exp. Biol. Med.* (USSR) *78:*17-19, 1974.
60. Stein, M., Schiavi, R. C., and Camerino, M.: Influence of brain and behavior on the immune system. *Science 191:*435-40, 1976.
61. Eurenius, K. and Mortensen, R. F.: The phytohemagglutinin (PHA) response in the thermally injured rat. *Int. Arch. Allergy 40:*707-18, 1971.
62. Monjan, A. A. and Collector, M. I.: Stress-induced modulation of the immune response. *Science 196:*307-08, 1977.
63. Folch, H. and Waksman, B. H.: The splenic suppressor cell. I. Activity of thymus-dependent adherent cells: Changes with age and stress. *J. Immunol. 113:*127-38, 1974.
64. Thaxton, P. and Siegel, H. S.: Modification of high temperature and ACTH induced immunodepression by metyrapone. *Poultry Sci. 52:*618-24, 1973.
65. Riley, V.: Mouse mammary tumor: Alteration of incidence as apparent function of stress. *Science 189:*465-67, 1975.
66. Dougherty, T. F. and Frank, J. A.: The quantitative and qualitative responses of blood lymphocytes to stress stimuli. *J. Lab. Clin. Med. 42:*530-37, 1953.
67. Van Dijk, H., Testerink, J., and Noordegraaf, E.: Stimulation of the immune response against SRBC by reduction of corticosterone plasma levels. *Cell. Immunol. 24:*8-14, 1976.
68. Durney, C. H., Johnson, C. C., Barber, P. W., et al.: *Radiofrequency Radiation Dosimetry Handbook,* 2nd ed. Report SAM-TR-78-22, 1978.
69. Lin, J. C.: Microwave Biophysics. In: *Microwave Bioeffects and Radiation Safety,* Stuckly, M. A., editor. *Trans. IMPI 8:*1554, 1978.
70. Bawin, S. M., Kaczmarek, L. K., and Adey, W. R.: Effects of modulated VHF fields on the central nervous system. *Ann. N.Y. Acad. Sci. 247:*74-81, 1975.
71. Blackman, C. F., Elder, J. A., Weil, C. M., et al.: Two parameters affecting radiation-induced calcium efflux from brain tissue. *Radio Sci.* (Suppl.) *14:*1979. In press.