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Immunologic and Cancer-Related Aspects of Exposure to Low-Level Microwave and Radiofrequency Fields

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INTRODUCTION

Microwave electromagnetic radiation (MW), 300–300,000 MHz (wavelength in air 1 m to 1 mm), and radiofrequency electromagnetic radiation (RF), 0.3–300 MHz (wavelength in air 1,000–1 m), are both relatively new but rapidly intensifying occupational and environmental factors. For about 40 years there has been a steadily increasing exposure of both occupational groups and the general population to various intensities of these radiations due to the use of MW/RFs in radar, navigation, communication, and television, as well as for multiple industrial and household purposes. Thus, biological effects and possible health hazards of MW/RFs have become an important problem to be solved in connection with the elaboration of valid safety standards. Despite numerous experimental studies and epidemiologic observations (1,2), and distinct philosophies of MW/RF safety standards in the U.S. and West European countries versus Eastern European countries, it is still not possible to prove the existence and character of specific molecular, cellular or system-related damages that may be evoked by exposure to low-level MW/RF fields. Most of the observed effects are inconsistent, transient and difficult to confirm and interpret. Absorption of a certain amount of electromagnetic energy in cells, biological tissues, and in living organisms results in a thermal load that cannot be dissipated to the environment. After exceeding the capacity of the thermoregulatory and adaptation mechanisms, it leads to an increase of temperature with all the known consequences of local or whole-body hyperthermia. Physiologic and pathologic effects of short-lasting MW hyperthermia have often been misinterpreted as being directly due to the influence of the radiation, and only recently, after progress in the measurement of specific absorption rates (SARs), the two phenomena have been differentiated.

The situation occurring in animals irradiated with MW/RFs is additionally complicated by the concomitant stress reaction. In subjects exposed to thermogenic fields, the stress reaction and the resulting well-known general adaptation syndrome can occur. But stresses related to low-level MW/RFs exposures may also occur. There exist anecdotal reports (3) that rodents (the main subject in MW/RF exposure studies) can perceive low radiation levels in an unknown way, and

that they seem to be aware of being irradiated in weak fields and try to escape from the irradiation area or at least move to areas with lower field intensity. Thus, possible behavioral effects due to discomfort caused by weak MW/RF fields may occur and in turn influence neurohormonal pathways. A typical stress reaction and adaptation syndrome with stimulation of the hypothalamo–hypophyseal–adrenal axis and release of catecholamines and adrenal steroids seem to be a reasonable consequence. These problems will be discussed.

The influence of non-thermal MW/RF fields on immune reactions and immune status, as well as on development and growth of neoplasms will be reviewed. Theoretically, these phenomena may be influenced both by stress and by possible specific effect of MW/RFs. There is general agreement that whole-body hyperthermia results in remarkable immunologic and cancer-related effects (4). Thus, when analyzing the relation of observed phenomena to MW/RF exposures, care must be taken to differentiate the three possibilities—specific interaction of MW/RFs at the molecular/cellular level, non-specific stress with adaptation syndrome, and possible thermal effects. Biological effects of MW/RFs at the cellular and subcellular levels were recently reviewed (5) with the general conclusions that no consistent changes in molecular or subcellular systems exposed *in vitro* can be attributed to specific MW/RF interactions. No consistent effects of these radiations have been demonstrated on growth and colony-forming ability of single cells, although there is an indication that sodium and potassium ion transport across red blood cell membranes can be affected in a manner different from generalized heating by exposure *in vitro*.

The possibility that specific cellular interactions of MW/RFs are connected with the pulse modulation of the carrier wave should be also considered. In Russian and East European literature of the 1960's and early 1970's (6,7) there exist different opinions concerning whether pulse-modulated MW radiation exerts stronger neurologic, behavioral, and immunologic effects compared with continuous-wave radiation of the same frequency. There is no convincing evidence that millisecond pulses of MW radiation at mean power densities not leading to detectable thermal effects may influence the function of living organisms to a higher degree than continuous-wave radiation. Certain cellular disturbances however, may be attributed to specific interactions of MW/RFs modulated at low frequencies (1–10 Hz). Adey and his group (8-10), on the basis of 20 years of experience in searching for cellular effects related to low-level sinusoidally-modulated MWs and RFs of different frequencies and pulse modulations in the low-frequency range, have found much evidence that the amplitude-modulation characteristics appear to be a prime determinant of the nature of interactions at the cellular level. The authors have stressed the existence of windowing in many of the observed interactions in both the frequency and amplitude (of pulses) domains; most of these interactions were connected with cell-membrane function (8). More recently, a series of enzyme responses as intracellular markers of events that are sensed at the cell membrane as a result of interactions with weak modulated MW/RF fields have been investigated (9,11). A strong inhibition of cAMP-independent protein kinases (messenger enzymes important for protein synthesis in the cell) occurred in cultured human lymphocytes exposed to a 450-MHz field, sinusoidally amplitude-modulated at 16 Hz (9).

The effect was strongly dependent on the modulation frequency, with diminishing responses at 40 and 60 Hz and no response at 80 or 100 Hz. The authors also observed a strong time-dependence for inhibition of protein kinases—the effect occurred only in the first 15 to30 minutes of exposure and disappeared thereafter, despite continued exposure.

These and other events occurring in cells exposed to low-level MW/RF fields are not fully understood or resolved. They indicate the complexity of the interactions that may be connected with the function of immunocompetent cells and the whole immune system, as well as with development and growth of neoplasms.

IMMUNOLOGIC RESPONSE TO LOW-LEVEL MICROWAVE AND RADIOFREQUENCY FIELDS

The complicated immune system provides a multifactoral non-specific and specific defense for the organism against various pathogens (bacteria and viruses), and protects against development of neoplasms. Besides the basic immunocompetent cells, originating from hemopoietic stem cells (lymphocytes, macrophage–monocyte system and granulocytes) many humoral factors (e.g., opsonins, complement system, interferons, interleukens) play an important role in non-specific and specific immunity. The immune system exhibits multistage internal cooperation and self-regulation based mostly on feedback mechanisms and extensive neurohormonal and endocrine control. The complexity of the immune system and its internal and external interrelations impede evaluation of immune functions under the influence of environmental factors. Some of these factors, including MWs and RFs, exert only inconsistent, non-specific and transient effects (12). Unfortunately, there exists no single test (or set of tests) that allows evaluation of the whole system, and thus most experiments are directed toward one or a few parameters of immunity. An integrated evaluation of immunity is possible on the basis of observations of course and/or final results (survival) of stabilized experimental bacterial or viral diseases, development of neoplastic tumors, or the widely used graft versus host reactions. Although such integrated evaluations do indicate immunosuppression or immunostimulation, they yield little information regarding the immunologic mechanisms underlying the observed changes in the host's resistance.

Another difficult problem involves the relation between the observed immunological responses and the investigated factor (Figure 1). Theoretically, MW and RF radiations may exert various specific effects directly on immunocompetent cells and their cooperative/regulatory mechanisms, or they may influence neurohormornal regulation of the immune system. Both the possible specific interactions of MW/RFs and the concomitant stress reaction trigger various adaptation mechanisms that may or may not be beneficial for the host's immunity. Because of adaptability and redundancy in the immune system and its regulatory mechanisms, the host can generally survive subtle and transient perturbations in single elements of immunity. Thus, the subtle effects generated by MW/RFs and the concomitant stress reaction may not lead to clinically detectable immune dysfunctions.

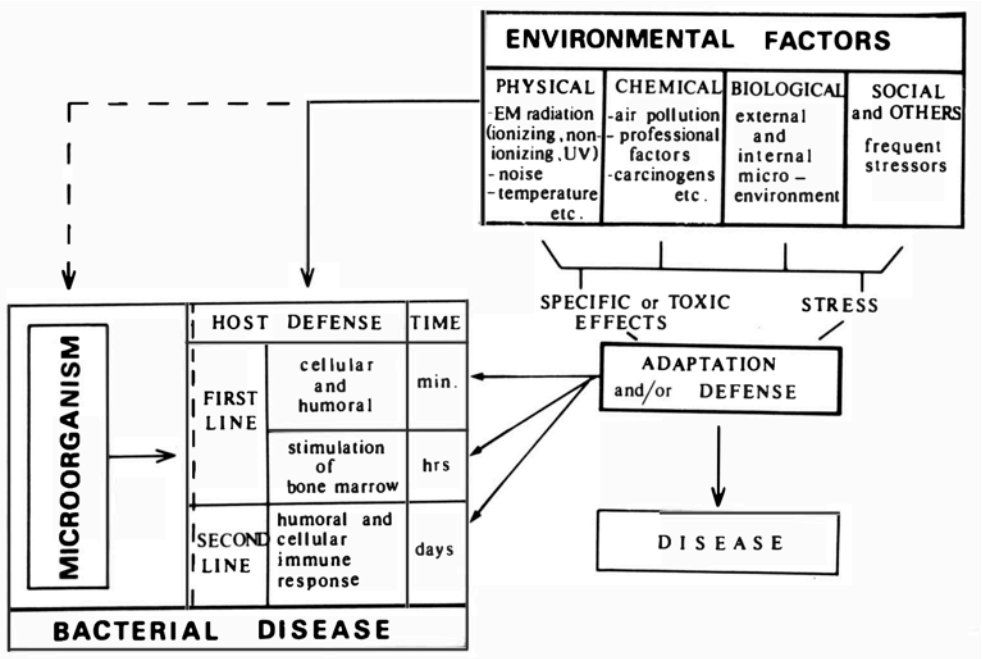

Figure 1. Possible interactions of environmental factors, including electromagnetic fields, with antibacterial resistance of the host. Environmental factors exert specific effects on various levels of biological organization (subcellular, cellular, organ, system), and a concomitant non-specific stress reaction. For defined factors the two elements of the biological reactions are expressed at certain levels (e.g., for electromagnetic radiation the stress reaction predominates). Specific effects and the stress reaction trigger adaptation and/or repair mechanisms, as a form of the host's defense against harmful effects. When the capacity of the adaptation mechanisms is exceeded, the factor-related disease (environmental disease, occupational disease) occurs. However, both the specific effect of the factor and certain elements of the triggered adaptation mechanisms (e.g., stimulation of hypophyseal–adrenal axis) may influence non-specific and/or specific immune reactions and result in increased susceptibility to bacterial infections (13).

In view of the above general comments, it is not surprising that the available literature on immunological responses to MW/RF radiations (4,12) does not offer convincing evidence for specific non-thermal interactions of the radiation with hemopoietic or immunocompetent cells. Animals exposed at different frequencies, modulations and power densities of MW/RFs using various facilities have shown inconsistent and transient changes in elements of the immune system (Tables 1–3) with the observed effects suggesting both immunostimulation and immunosuppression or inconsistent changes. In view of the large amount of conflicting and sometimes misleading information on the effects of MW/RF radiation on the immune system, we have grouped the data as follows: alterations in immunocompetent cells irradiated *in vitro*; responses to low-level short-term exposures *in vivo*; response to low-level long-term exposures *in vivo*; and integrated evaluation of immunity in MW-exposed animals.

Table 1. Alterations of Immunocompetent Cells Exposed to Microwave Fields *In Vitro*

Observed Effects	Type of Cells	Conditions of Exposure	Reference	Remarks
Increased spontaneous blastic transformation of lymphocytes	Human lymphocytes	3000 MHz, PM, 4 hr daily, 3–5 days at 7 mW/cm^2	(14)	Effect not confirmed. SAR not specified, field measurement doubtful
No increase of spontaneous or mitogen-induced blastic transformation of lymphocytes exposed to non-thermal MW fields	Murine splenic rat lymphocytes	2450 MHz, CW, 1– 4 hr 10 mW/cm^2, SAR 10 mW/g 2450 MHz CW, 1–4 hr 5–20 mW/cm^2	(15)	No increase of medium temperature
Increased spontaneous blastic transformation of lymphocytes at elevated temperatures	Human murine lymphocytes	Water bath	(16-19)	Maximal transformation at 39°C; rapid inhibition of transformation above 41°C
No change in viability or growth	Human lymphoblasts (cultured)	2450 MHz CW, waveguide, 15 min 10–500 mW/cm^2	(20)	
Depressed phagocytosis	Murine peritoneal macrophages	2450 MHz, 50 mW/cm^2 30 min with cooling of medium	(21)	Effect not confirmed. Possible microthermal cell injury
Release of lysosomal enzymes, lowering of viability	Rabbit granulocytes (isolated)	3000 GHz PW, 15–60 min 1–5 mW/cm^2 (SAR not determined)	(22)	Field measurements doubtful, possible thermal effects
Suppression of T-lymphocyte cytotoxicity	Murine lymphocytes	450 MHz, modulated at 60 Hz, 1 mW/cm^2, 4 hr	(10)	Effect depends on modulation, windowing of the effect
Lowering of protein kinase activity	Human tonsil lymphocytes	450 MHz, modulated at 3–100 Hz, 1.5 mW/cm^2, 15–60 min	(9)	As above, maximal effect at 16–60 Hz

Table 2. Immunological Responses to Short-Term Exposures to Microwaves *In Vivo*

Observed Effects	Species	Conditions of Exposure	Reference	Remarks
Increased antibody levels to SRBC, reversible increase in proliferation of lymphocytes	Mice	3000 MHz PW, 0.5–5 mW/cm^2, 3 hr/day, 6 weeks	(23)	SAR not specified, field measurement doubtful
Transient enhancement of cell mediated and humoral immunity	Mice	2450 MHz, 10 mW/cm^2 4 hr/day, 4–5 days	(24)	
Increased circulating antibody response to SRBC	Mice	9000 MHz PW, 10 mW/cm^2 2 hr/day, 5 days	(25,26)	At 1 mW/cm^2 negative findings, threshold below 10 mW/cm^2
	Mice	3000 MHz PW, 1–12 mW/cm^2 1 hr/day, 2 days	(27)	Maximal effect at 7 mW/cm^2; threshold between 1–5 mW/cm^2
No detectable effects after short-term exposures in non-thermal MW/RF fields	Mice	2450 MHz CW, 5–35 mW/cm^2 1–22 days, 15–30 min/day	(28)	
	Rats	100 MHz CW, 46 mW/cm^2, up to 57 days, 4 hr/day	(29)	
		2880 MHz PW, 5–10 mW/cm^2, 3–7.5 hr/day	(30,31)	
		Responses to Brief Microwave Hyperthermia		
Increased number and faster maturation of B lymphocytes in spleen; Enhanced response to B lymphocyte mitogens	Mice	2450 MHz, wave-guide, SAR 14 mW/cm^2, 30 min or 3 time	(32,33)	Threshold at about 12 mW/cm^2, effect strain-specific for mice
	Mice	2450 MHz CW, anechoic chamber, 40 mW/cm^2, 30-min single session	(34)	
Increase in number of T lymphocytes without change in B cells	Mice	2450 MHz, 15 mW/cm^2 30 min/day, 9 days	(35)	
Increase of T and B lymphocytes in spleen, reduction in lymphocyte distribution	Mice	2600 MHz, 15 mW/cm^2 15–60 min	(36,37)	Similar effects after administration of glucocorticoids
Suppression of non-specific cell mediated and humoral immunity	Mice	2450 MHz, CW, 30 mW/cm^2, 2–9 sessions	(38)	
	Hamsters	2450 MHz, 40 mW/cm^2, 60 min/day, 1–5 days	(39)	Similar effect after glucocorticoids

Observed Effects	Species	Conditions of Exposure	Reference	Remarks
Increase of macrophage phagocytosis and viricidal capacity	Mice Hamsters	As above	(38,39)	
Increase of macrophage cytotoxicity to virus-infected cells	Hamsters	2450 MHz, 25 mW/cm^2, 60 min/day, 1–5 days	(40,41)	
Increased survival of herpes infections (encephalitis)	Mice	2450 MHz, 40 mW/cm^2, 2 hr/day, 8 days	(42)	
Stimulation of cell-mediated immunity, followed by transient suppression	Mice	2450 MHz, 40–60 mW/cm^2, 2 hr/day, 1–14 days	(4,43)	Stimulation after 1–4 days of hyperthermia, suppression after 10–14 days of exposure

Table 3. Immunological Response to Low-Level Long-Term Exposures to Microwave Fields *In Vivo*

Observed Effects	Type of Cells	Conditions of Exposure	Reference	Remarks
Lowered circulating antibody levels to *Salmonella*	Rabbits, Mice, Guinea pigs	10 GHz CW, 10 mW/cm^2, few months	(44)	Methods and conditions of exposure not described in detail
No detectable effects, including responsiveness of lymphocytes to mitogens	Rats	2450 MHz CW, 5 mW/cm^2, 15–30 min/day, 22 days	(28)	Relatively short period of exposure
Transient suppression of phagocytic capacity of granulocytes, suppressed cell-mediated and humoral immunity	Rats	2450 MHz, 0.5 mW/cm^2, 1–3 months, daily exposures not specified	(45)	
Slight suppression of B-lymphocyte reactivity	Rabbits	2450 MHz, 10 mW/cm^2, 23 hr/day, 6 months	(46)	Results obtained in small number (4) of rabbits
No detectable changes in blood picture and reactivity of lymphocytes to mitogens	Rabbits	2450 MHz CW, 0.5–5 mW/cm^2, 23 hr/day, 90 days	(47)	
	Rats	2450 MHz CW, 0.48 mW/cm^2, 23 hr/day for life span	(48)	
Increased susceptibility to staphylococcal infections with lowered phagocytic capacity of macrophages	Mice	2450 MHz CW, 5–15 mW/cm^2, 6 or 12 weeks, 2 hr/day	(13)	Transient changes, effects after 12 weeks exposure
Accelerated development of neoplasms, lowered natural antineoplastic resistance	Mice	2450 MHz CW, 5–15 mW/cm^2, 1–6 months, 2 hr/day	(49,50)	

ALTERATIONS IN IMMUNOCOMPETENT CELLS IRRADIATED *IN VITRO*

Several studies (Table 1) have attempted to determine whether *in vitro* exposure of lymphocytes and other immunocompetent cells to MW/RF radiations at various intensities leads to metabolic or functional alterations in these cells. In an early study, often cited in the literature, Stodolnik-Baranska (14) exposed unstimulated cultures of human lymphocytes to 3,000-MHz pulse-modulated (millisecond pulses) MWs for 3–5 days at 7 mW/cm^2 (4 hours daily) or 14 mW/cm^2 (15 minutes daily). The MW-exposed cells exhibited a fivefold increase in lymphoblastoid transformation, as measured morphologically by counting the number of blastoids (transformed small lymphocytes with active synthesis of nucleic acids and proteins), compared with unexposed controls showing weak spontaneous transformation. The intriguing observation was, however, never confirmed despite many attempts performed under better controlled conditions of irradiation, and with the use of more objective methods for evaluation of blastoid transformation of lymphocytes (15,23,51). On the other hand, numerous studies have shown an increased mitogenic response of human blood lymphocytes in culture after temperature elevation above 37°C (16,17,52). Human lymphocytes cultured at 38–40°C respond with faster and increased blastoid transformation both in unstimulated cultures (elevated temperature acts as a transforming factor), and in cultures stimulated with phytohemagglutinin (PHA) or concanavalin A (ConA), the two mitogens commonly used for studies of lymphoproliferative response *in vitro*. Roszkowski et al. (52) found that maximal transformation of human lymphocytes occurred at 39°C, and that above 41°C there was inhibition of lymphocyte function and an unresponsiveness to PHA and ConA. A similar blastogenic response was found when cultures were heated to 39°C in a water bath, or after exposure to 2450-MHz MW field (unpublished results). Inhibition of the transformation occurred above 41°C, independently of the source of thermal energy.

Smialowicz (15) exposed splenic murine lymphocytes to 2450-MHz continuous-wave radiation for 1–4 hours at 10 mW/cm^2 (SAR, 19 mW/g). Immediately after completing the irradiation session the temperature of the exposed cultures did not differ from that of the controls, and the viability of the cells was unchanged. Following irradiation, the exposed and control cultures were treated with non-specific T and B lymphocyte mitogens (PHA, ConA, lipopolysaccharides), and the proliferative response was measured after 72 hours by incorporation of ^{3}H-thymidine. The blastogenic response of MW-irradiated and sham-irradiated cultures did not differ in cultures stimulated with mitogens or in unstimulated cultures. In a similar experiment, Hammrick and Fox (51) exposed rat lymphocytes to 2450-MHz continuous-wave radiation for 4, 24, or 44 hours at 5, 10 and 20 mW/cm^2 (SARs of 0.7, 1.4 and 2.8 mW/g, respectively) and measured the transformation of unstimulated and PHA-stimulated cultures by incorporation of ^{3}H-thymidine. These investigators also did not observe an influence of MW irradiation on blastic transformation of lymphocytes. The effect of the same frequency of MW radiation on the growth and viability of cultured human lymphoblasts was studied by Lin and Peterson (20). Continuous cultures of human lymphoblasts (established cell lines Daudi and HSB_2) were irradiated in a wave-guide facility for 15 minutes at 10–500 mW/cm^2 (SARs of 25–

1200 mW/g). Due to cooling of samples kept during irradiation in capillary tubes, no increase in temperature was observed, even at the highest power densities tested. No changes were observed in the viability and growth of MW-exposed lymphoblasts compared with unexposed controls.

The above studies, performed under well-controlled conditions of exposure, provide evidence that no detectable changes in lymphocyte activity occurs following MW exposure *in vitro* when proper control of culture temperature is achieved and no elevation of the medium temperature occurs. Thus, the original observation of Stodolnik-Baranska (14) cannot be related to specific, non-thermal influence of MW radiation and must be due to other causes. In the original experiments, performed using facilities at the Institute of Aviation Medicine in Warsaw, Poland, the following conditions were present: 3000 MHz millisecond pulse MW generator; open field from horn antenna; absorbing screen opposite the antenna; no anechoic chamber. No measurement of field power density during irradiation was made; instead, an estimation of the power density based on earlier measurements was given. The lymphocyte cultures were irradiated in thick-walled, glass culture vessels. Under the above conditions, absorption of MW energy inside the vessels was undeterminable, and there also existed a possibility of large difference of power density compared with the listed levels of 7 or 14 mW/cm^2. It is reasonable to assume that the temperature of the medium was elevated during irradiation, and that the hyperthermia resulted in stimulation of blastic transformation of the irradiated lymphocytes.

Other immunocompetent cells have rarely been the subject of experiments with *in vitro* exposure to non-thermal MW/RF fields. Information concerning the influence of this radiation on viability, function, and morphology of macrophages, monocytes and granulocytes is scarce and fragmentary. In an early study, Mayers and Habeshaw (21) found depressed phagocytosis of isolated peritoneal murine macrophages exposed to 2450 MHz at 50 mW/cm^2. During irradiation, a 2.5°C increase of medium temperature was noted, however the final temperature in the culture vessel did not exceed 36.2°C. The suppressed phagocytic activity returned to normal after discontinuation of MW irradiation. The mechanisms by which the observed effect occurred are unknown. Such a slight increase in temperature normally results in stimulation, and not suppression, of phagocytosis; this process is optimal for human macrophages at 38–39°C and for murine peritoneal macrophages at 39–40°C.

More recently, Rama Rao et al. undertook broad investigations on the effects of MW exposure on the hamster immune system (40,41). Since the animals were irradiated *in vivo* (2450 MHz, 25 mW/cm^2, 60 minutes) the results will be discussed in detail in the next section, however it is worthwhile to mention here that peritoneal macrophages isolated from the irradiated hamsters were found to be activated, as measured by their viricidal activity to vaccinia viruses (40).

Szmigielski (22) exposed isolated rabbit granulocytes *in vitro* to continuous 3000-MHz MW radiation at 1 and 5 mW/cm^2 for 30–60 minutes and observed an increased number of dead cells (increase in nigrosine staining), and an enhanced liberation of lysosomal hydrolases (a symptom of sublethal cell damage). Since the same facility, used by Stodolnik-Baranska (14) and Czerski

(23) (described above) was used for this study, there is no certainty regarding the field power density, and significantly higher intensities than those reported were possible. Unfortunately, the experiments with granulocytes were never repeated under more controlled exposure conditions.

In summary, there is no convincing evidence for metabolic and/or functional alterations in immunocompetent cells irradiated *in vitro* in non-thermal MW/RF fields. Thus, the reported immunologic phenomena observed in animals exposed *in vivo* must be explained on the basis of alterations of humoral control and/or regulatory mechanisms of the immune system or nonspecific stress reaction of the irradiated subjects.

Our present knowledge of non-thermal effects of MW/RFs on the function of immunocompetent cells is still scarce and fragmentary. Only simple experimental systems have been tested, and specific functions of immunocompetent cells have only rarely bee n investigated. Virtually nothing is known about the influence of RFs (0.3–300 MHz) on these cells, and on effects related to pulse modulation of the carrier wave. We have mentioned earlier that Adey and his group (8) have evidence that pulse and amplitude modulations of the carrier wave appear to be a prime determinant of the nature of interaction at the cellular level. Recently this group reported suppression of T-lymphocyte cytotoxicity following exposure to sinusoidal, amplitude- modulated 450-MHz MW fields (10), and alterations in protein kinase activity following exposure of cultured human lymphocytes to MW fields of the same frequency and modulation (9). In both experiments, well-controlled conditions of irradiation and measurements were provided (anechoic chamber for 450-MHz MWs, a Crawford cell exposure) and described in detail (10). The incident power densities were relatively low (1.0–1.5 m W/cm^2) with sinusoidal amplitude modulation at 3–100 Hz. Murine cytotoxic T lymphocytes (line CTLL-1) exhibited a significant inhibition of allogeneic cytotoxicity against the target cells MPC-11, when a 4-hour assay of cytotoxicity was conducted during irradiation in a 450-MHz field sinusoidally amplitude-modulated at 60 Hz. Exposure of the effector cells to the same field prior to adding them to target cells resulted in a similar inhibition of the cytolytic assay (10). This suggested a direct interaction of the field with the function of cytolytic T lymphocytes. The exposed cytolytic T cells recovered their full cytotoxic capacity about 12 hours after termination of exposure. Still more interestingly, a differential susceptibility of the cytolytic T cells was observed with modulation frequencies below 100 Hz. Peak suppression occurred at 60 Hz modulation, with progressively smaller effects at 40, 16 and 3 Hz, while the unmodulated carrier wave (450 MHz) did not affect the cytotoxicity in any way. An identical exposure system and similar conditions of irradiations were used (9) to study the influence of 450-MHz MWs on the activity of certain enzymes in human tonsil lymphocytes irradiated *in vitro*. In these experiments, lymphocytes were kept in the Crawford cell housed in a temperature-controlled chamber (35°C) and irradiated for periods up to 60 minutes. It was found that the activity of cAMP-dependent protein kinase relative to controls remained unaltered by 450-MHz fields modulated at 16 or 60 Hz with exposures of 15, 30 and 60 minutes. On the other hand, total cAMP-independent protein kinase activity fell to less than half of that of the unexposed control levels after 15 and 30 minute exposures. Surprisingly, it returned to control levels during continued exposure for 45 and 60

minutes. The reduced enzyme activity occurred with 16, 40 and 60-Hz modulation frequencies, but not with 3, 6, 80, or 100-Hz modulations.

The biological significance of the large reduction in histone kinase activity of the lymphocyte during exposure to very low and definitely non-thermal (1.0–1.5 mW/cm^2) levels of modulated MW radiation is unknown, but it points out the windowed character of the response regarding both the low modulation frequencies and the time of irradiation.

Adey and his group ((8,11) and personal communications) offer a general explanation and hypothesis for the cellular events that occurred after exposure to the weak, modulated MW fields. They presumed, on the basis of numerous experimental studies, that the primary interactions occurred at the cell membrane level, and they concluded that there were three basic steps in the sequence of events at the cell membrane. First, there was a modification of calcium binding that occurred along the membrane surface, this being a highly cooperative step and appearing to be the basis of the amplification that occurred in the response to weak electromagnetic stimuli. The second step was the coupling of the signal across the cell membrane. The electromagnetic waves (depending on their modulation) might have been involved as the vehicle for passing the signal down the strands of helical proteins that span from the outside to the inside of the membrane. The third stage involved coupling to intracellular systems (coupling to the cytoskeleton and activation of intracellular enzymes, either directly at the membrane or indirectly through chemical signaling).

This hypothesis, if proved in more detail and supported by further experiments elucidating principles of the windowing in interaction of weak electromagnetic fields at the subcellular and cellular levels (relation to pulse modulation, time of exposure and frequency of the carrier wave), may change significantly the views on biological effects of MW/RFs, including influence of this radiation on immunocompetent cells.

IMMUNOLOGIC RESPONSE TO LOW-LEVEL SHORT-TERM EXPOSURES *IN VIVO*

Over the past 30 years many reports have appeared (1) dealing with various effects of MW/RF radiation on immunologic functions of irradiated animals. In many earlier cases (1955–1975) the investigators were motivated by the search for possible harmful effects on the immune system, supporting the philosophies of safety standards elaborated in their countries. Thus, when analyzing the literature of the 1960s and early 1970s, one is confronted with different methodological approaches and different conditions of irradiation used by Western and Eastern authors. As a rule, Russian and East European authors searched for any detectable shift in hematologic and/or immune systems after exposure of animals to weak (below 1 mW/cm^2) and very weak (10–100 $\mu W/cm^2$) fields, and related the observed transient shifts to action of MW/RF radiation. West European and American investigators concentrated on power densities leading to subthermal or definitely thermal effects in the organism (10 mW/cm^2 and above). However, the findings due to local or whole-body hyperthermia (even that lasting only a few minutes) were often misinterpreted as being related to the interaction of MW energy with the function of the

immune system. In any case, the final interpretation of MW/RF-induced changes in the immune system, and conclusions regarding possible causative relations must consider many variables that may affect the interaction of electromagnetic radiation with the biological system (12). Electromagnetic radiation generally evokes weak, transient, and inconsistent biological effects. In contrast, whole-body hyperthermia is a strong stimulus for the immune system and, as it is well-known in the case of fever, it modulates the function of this system. Thus in experiments involving animals in weak MW/RF fields, sham-irradiated controls should be used, and care should be taken to protect the animals against stresses from handling. In the case of thermogenic MW/RF fields, hyperthermic and stress control groups (positive controls) must be considered. From personal experience and autopsies in numerous laboratories in the Soviet Union, Poland and Czechoslovakia, the present authors can state that up to 1975 the conditions of exposure of the animals, field measurements, and the general handling of the experimental animals were far from being what is presently regarded as acceptable. As a rule, cage-controls were used instead of sham-irradiated controls, and in many experiments generally poor animals were used and were improperly handled. Thus, in our opinion, the earlier Russian and East European investigations involving animals exposed to weak MW/RF fields (6,7,44), including the reported immunological alterations, should be viewed with caution. In the most recent Russian monograph on biological effects of electromagnetic fields (53), immunologic alterations in mice and rats exposed to MW fields at very low power densities (reported repeatedly during 1960–1975) are not presented. Instead, the authors claim that inconsistent and transient immunological alterations may be observed in animals chronically exposed at power densities exceeding 0.5 mW/cm^2, and that clearly demonstrable immunological effects are observed only at hermogenic power densities. The authors also state, although without evidence, that the “high adaptability of the immune system causes the alterations observed after exposure of animals in weak MW/RF fields; they are meaningless from the point of view of safety standards” ((53), p.55).

The situation has changed during the last decade. Several laboratories in the USSR and East European countries were equipped with anechoic chambers, modern devices for measurements of power density and SAR, and better handling and care of animals were provided. This is still not the rule everywhere.

In investigations published in 1972–1976 (6,54) transient and reversible increased lymphocyte proliferation and function, as well as activation of single subpopulations of lymphocytes following exposure to subthermal MW/RF fields, were believed to be the most consistent findings. Recent data (Table 2) indicate that immunologic function in a wide range of animal species may be altered by single or short-term (several days) exposure to MW fields; however, the effects are inconsistent and not detrimental for the organism.

Transient enhancement of cell-mediated and humoral immunity, measured with a battery of tests including splenic plaque-forming cells, ability for phagocytosis, and responsiveness to sheep red blood cells (SRBC), were found in mice in a series of experiments performed under

well-controlled conditions of irradiation (4 hr/day, 2450 MHz, 10 mW/cm^2) by Roberts and Steigbeigel (17), and by Ivanoff et al. (24). Liddle et al. (26) reported stimulation of the circulating antibody response (against streptococcal type III polysaccharide) in mice exposed to pulsed (pulse repetition rate about 1000 per second) 9000-MHz MWs at 10 mW/cm^2 (SAR, 4.7 mW/g), 2 hours daily for 5 days, with a concurrent lengthening of the survival time of animals challenged following exposure. More recently, the authors performed a similar experiment at 1 mW/cm^2 (25). They found that the circulating antibody titers for the MW-exposed animals were not significantly different from those of the sham-irradiated animals, and that there were no differences in any of the hemato-immunological parameters analyzed, indicating that the threshold in mice for the response was 1–10 mW/cm^2 (0.47–4.7 mW/g). Similar results were reported recently by Chinese investigators (27). These authors exposed mice to pulsed 3000-MHz MWs (from a radar generator) for 1 hour daily during two consecutive days, and found a fourfold increase of the hemagglutinin titer to SRBC 7 days after termination of exposure, with a return to normal values 22 days after irradiation. In another set of experiments (27) the same investigators searched for a threshold for the observed phenomenon. Mice were irradiated under identical conditions except that 1, 5, 7, and 12 mW/cm^2 (SARs not given) were applied. A maximum increase of the hemagglutinin titer to SRBC was observed after exposures at 7 mW/cm^2, but the titers were also significantly elevated compared with sham-irradiated controls at 5 and 12 mW/cm^2. At 1 mW/cm^2 the hemagglutinin titer did not differ from controls, and thus the authors concluded that for pulsed 3000-MHz MWs, the threshold for the phenomenon was 1–5 mW/cm^2.

In an earlier study, Czerski (23) reported that mice exposed for 6 weeks to 2950-MHz pulsed MWs (from a radar generator) at 0.5 mW/cm^2 (SAR not determined), 12 hr/day, had significantly greater numbers of antibody-producing cells (lymphoblastoids and large lymphocytes) and higher serum hemagglutinin titers following immunization with SRBC. Cage-controls were used, and the facilities available did not permit precise measurements of the field power density in the area of irradiation of the animals. Wiktor-Jedrzejczak et al. (55,56), using facilities available at the Naval Medical Research Institute in Bethesda and at the FDA Bureau of Radiological Health in Rockville, MD (a rectangular waveguide), exposed mice to 2450-MHz MWs for 30 minutes at a SAR of about 14 mW/g (thermal effects very possible). After one irradiation session, a significant increase in the proportion of B lymphocytes (type of lymphocytes responsible for humoral immunity) was found based on determination of surface receptors (CR, Ig, Fc) (typical for B lymphocytes at various stages of maturity). This was accompanied by enhanced response of murine spleen lymphocytes to the B-cell specific mitogens (lipo-polysaccharides, polyinosinic-polycytydylic acid, and purified protein derivative of tuberculin). In contrast to the other above-cited experiments, Wiktor-Jedrzejczak et al. (55) found a decrease, not an increase, in the primary humoral response to SRBC in mice exposed to a single session of 2450-MHz MWs with a SAR of about 14 mW/g. However, in this case the animals were immunized just prior to the exposure and not after termination of exposures as was the case in the investigations discussed earlier. The authors suggested that the decreased humoral

response to SRBC observed in their experiment might have been due to nonspecific stimulation of B lymphocytes by MW exposure to mature before they were activated by the antigen (SRBC). This could have resulted in increased proportion of unresponsive lymphocytes.

The investigations were continued by Sulek et al. (57) using the same facilities. They investigated the kinetics for increased frequency of CR-positive cells (B lymphocytes) in the spleens of mice irradiated as before, and determined the threshold for the observed phenomena. It was found that the frequency of CR-positive cells showed an initial increase 3 days following a single session of 2450-MHz exposure (30 minutes) at a SAR of 12 mW/g, and that it persisted for 5–6 days. A minimum of 15 minutes exposure at a SAR of 11.8 mW/g or 30 minutes at 5 mW/g were needed to cause a significant increase in the number of CR-positive cells in murine spleen. The number of T lymphocytes was unchanged. In contrast, Huang and Mold (35) found a significant increase in number of T lymphocytes without changes in B lymphocytes in mice exposed to 2450-MHz MWs at 15 mW/cm^2 (SAR 10 mW/g) for 30 minutes during 9 days. Schlagel et al. (32) and Smialowicz et al. (34) in two independent investigations re-examined the phenomenon of shifts in murine spleen lymphocyte subpopulations after exposure to 2450-MHz MW fields. It was found that the observed increase in number of CR-positive and Fc-positive lymphocytes was strain-dependent, and that it occurred only in mice of a certain age (detectable in 16-week old animals, but not in younger animals). The exposure facilities used (waveguide versus anechoic chamber), as well as the environmental conditions were additional sources of variation that might have influenced the appearance of this phenomenon (34).

In fact, all the observed shifts in murine spleen lymphocyte populations were due to thermal stress induced by exposure to MW fields. Evidence for this view has come from studies of Liburdy (36,37), who has found similar shifts in splenic lymphocytes in mice exposed to 2600 MHz at 800 mW/cm^2 (SAR, 5.6 mW/g) that resulted in a 2–3°C increase in rectal temperature. Similar phenomena were observed after administration of glucocorticoids, suggesting that the observed shifts might also have been related to some form of non-specific stress. In another set of experiments Liburdy (37) exposed mice to 2600 MHz MWs for 1 hour at 5 or 25 mW/cm^2 (SARs of 3.8 and 10 mW/g, respectively) and used two positive controls (injection of glucocorticoids and exposure of animals in a 63°C warm-air oven for 1 hour) as well as sham-irradiated mice. Prior to exposure, all animals were injected with ^{51}Cr-labelled syngeneic spleen cells with the aim of examining the distribution of these cells in the lung, liver, spleen and bone marrow. A changed distribution and migration of lymphocytes was observed in mice exposed to 2600-MHz MWs at 25 mW/cm^2 (a 2°C increase in body temperature) and in the glucocorticoid-treated groups, while no changes were found in animals exposed to 5 mW/cm^2 (no detectable increase of body temperature).

Further evidence for an influence of moderate-strength MW/RF-induced brief thermal stress on the function of the immune system was reported in 1983–1985 by Smialowicz et al. (38), Cain, et al. (40,41,58), and Yang et al. (39). Brief exposure to thermal 2450-MHz MW fields resulted in transient suppression of non-specific cell-mediated cytotoxicity (NK cell activity),

and a concomitant increase in macrophage phagocytic activity. Smialowicz et al. (38) exposed mice in anechoic chambers to 2450-MHz MW fields at SARs of 3.5, 10.5, or 21 mW/g for 90 minutes, and found a significant reduction of splenic NK cell activity, as measured using *in vivo* and *in vitro* assays, only at the highest SAR. NK activity returned to normal 24 hours following the last exposure at 21 mW/g; treatment of mice with hydrocortisone resulted in a similar suppression of NK activity. Rama Rao et al. (40,41) and Yang et al. (39) exposed hamsters to 2450 MHz at 5–40 mW/cm^2 (SAR of 0.53 mW/g per mW/cm^2), Each irradiation session lasted 1 hour, and the protocol included exposures of 1–5 sessions. Exposure at 25 mW/cm^2 (SAR of 13 mW/g) resulted in an increase of rectal temperature of 3.0–3.5°C at the end of irradiation; the temperature returned to normal 1 hour after termination of exposure. Exposure to 25 mW/cm^2 (single 1-hour session) induced a marked but transient alteration in body temperature, serum glucocorticoid levels, circulating leukocyte profile and NK cell activity. In contrast, after exposure at 15 mW/cm^2 no detectable changes of these parameters were found compared with sham-irradiated controls, and the authors concluded that the suppression of NK activity was related to thermal stress.

In another set of experiments performed under the same conditions of MW exposure, the same authors (40,41) found that exposure at 25 mW/cm^2 (rectal temperature increase, 3.0–3.5°C) resulted in activation of peritoneal macrophages that were significantly more viricidal to vaccinia viruses, compared with sham-irradiated and cage-controls. Moreover, immune macrophage cytotoxicity for virus-infected and non-infected target cells *in vitro* was not suppressed, indicating that peritoneal macrophages were not functionally injured by microwave hyperthermia. The above phenomenon did not occur after exposure at 15 mW/cm^2 (no detectable increase of body temperature).

In summary, the only repeatable effect on the immune system that might be attributed to short-term exposure of experimental animals at non-thermal power densities (SAR below 6–12 mW/g for mice) seems to be the enhanced humoral response to various antigens (SRBC, bacterial polysaccharides). Increased levels of circulating antibodies were found by a few independent groups of investigators, and the threshold for this phenomenon in mice seems to be 1–10 mW/cm^2—power densities not related to thermal stress. The mechanisms underlying the increased levels of circulating antibodies are unknown. Recently Rama Rao et al. (58) investigated the effect of a single 1-hour exposure of hamsters to 2450-MHz MWs at 5–15 mW/cm^2 on the IgM antibody response of spleen cells to SRBC, using the direct hemolytic plaque assay. Although most of the observed effects were related to thermal fields, exposure of hamsters at 15 mW/cm^2, not leading to detectable hyperthermia, also resulted in a significant increase of plaque-forming cells. The authors concluded that MW exposure augmented the primary IgM response to SRBC by affecting some early event in the immune response process. Despite the possible mechanisms leading to enhanced humoral response in MW-exposed animals, the biological significance of this phenomenon is uncertain. In fact, short-term exposures in subthermal MW fields act as an adjuvant for various antigens, and thus should be considered beneficial for the organism.

All other reported immunological responses occurring after short-term exposure to MW fields (e.g., shifts in distribution of lymphocytes, changes in number and reactivity of B lymphocytes, suppression of cytotoxic activity of the NK type, activation of macrophages) are related either directly or indirectly to thermal load and/or the concomitant stress with release of adrenal steroids. An interesting possibility based on the results of Rama Rao et al. (58) is that the cause of the immunologic responses observed after short-term thermal MW stress may be the release of endotoxins into the circulation. Endotoxin is a known B-lymphocyte mitogen which causes a polyclonal B-cell stimulation with increased production of antibodies, and which stimulates a number of macrophage functions including their anti-viral, antineoplastic and antibacterial activities. It evokes most of the phenomena observed after short-term MW thermal exposures (single or repeated during a few days). This also may be beneficial for the organism with potential therapeutic use. In fact, mice infected experimentally with herpes or vaccinia viruses and treated with 2-hour sessions of 2450-MHz MW hyperthermia have shown better tolerance of the infections and significantly higher survival of herpetic encephalitis (42). One must, however, remember that prolongation of exposures to moderate MW hyperthermia for several days results in a dramatic shift from stimulation to suppression of immune reactivity, as was clearly demonstrated by our group (4,13,43) in experiments in which mice were exposed to 2450-MHz MWs at 40 mW/cm^2 (SAR of 20 mW/g) 2 hours daily for 1, 4, 7, 10 or 14 days. Stimulation of immune reactions was observed only following 1- and 4-day exposures, and was followed by deep, although reversible, suppression of immune reactivity.

RESPONSE TO LOW-LEVEL LONG-TERM EXPOSURES

Short-term exposures to low-level MW/RFs do not cause harmful effects on the immune system, and thus to search for possible health hazards it is necessary to consider the response to long-term exposures (few weeks to several months for experimental animals, up to several years for humans exposed occupationally or environmentally to MW/RF fields). In cases of long-term exposures, problems of valid control groups and proper conditions of irradiation (environmental control of exposure chambers, avoidance of immobilization, isolation or confinement of animals during irradiation, careful handling of animals, etc.) becomes crucial for obtaining valid results that may be related to the influence of radiation. It must be stressed that the above conditions were not strictly followed in earlier investigations, mainly those performed in the USSR and East European countries, from which came most of the reports of the 1960's and early 1970's indicating immunological responses occurring after long-term exposures to weak MW/RF fields. Thus, despite the fact that there exist numerous publications supporting alteration of function of the immune system after long- term exposure at power densities below 0.5 mW/cm^2 (Table 3), at least some of the observed phenomena seem to be due to different stress situations resulting from normal handling, and from poorly controlled irradiation conditions. Great care must also be taken to avoid incidental bacterial and/or viral infections of the experimental animals exposed for several weeks to daily sessions of irradiation. Infections can easily occur under inadequate breeding conditions, and they are difficult to recognize and diagnose (especially if their course is

chronic or subclinical). Undoubtedly, such infections would alter the function of the immune system. We feel that the problem of incidental chronic and/or subclinical infections occurring during investigations of long-term exposures to MW/ RF fields is presently underestimated. In most of the long-term experiments performed under acceptable conditions of irradiation and animal care, various effects on immunosuppression have been found (Table 3). The susceptibility of animals to incidental infections should therefore be increased, with consequent further immune-system reactions not related directly to MW exposure.

In earlier experiments (44) Russian investigators found a reduction of circulating antibodies to *Salmonella* in mice, rabbits, and guinea pigs immunized following several months of daily exposures to 10,000-MHz MWs at 10 mW/cm^2 (SAR not stated). Unfortunately, the conditions of irradiation, time of daily sessions, and even the period of exposure were not described. Most of the acceptable information on immunological responses to low-level long-term exposure of experimental animals to MW/RF radiations have come however from investigations performed during the last decade.

Smialowicz et al. (28) exposed mice to 2450-MHz MWs under far-field conditions for 15 or 30 minutes daily for up to 22 days at 5–35 mW/cm^2 (SAR of 4–25 mW/g), and investigated various immunologic functions, including *in vitro* mitogen stimulation of isolated splenic lymphocytes, proportion of T and B lymphocytes in the spleen, and the primary humoral response to SRBC. They found no differences in the immunologic parameters compared with sham-irradiated controls, but the period of irradiation was relatively short (up to 22 days). Transient immunosuppression was observed in rats and rabbits exposed during a few months to 2450-MHz MWs in parallel American-Russian investigations (45,46) (Table 3). McRee et al. (59) reported that 1 month after termination of a 6-month exposure to 2450-MHz MWs at SAR of 1.5 mW/g (23 hours daily), spleen cells from rabbits showed a decreased responsiveness to pokeweed mitogen (a specific mitogen for B lymphocytes). Smialowicz (12) commented however, that "although these results are interesting, they are not conclusive and are of questionable value, because only four exposed and four sham-irradiated rabbits were employed. Also, both irradiated and sham-irradiated rabbits were transported from one laboratory to another (University of Washington to Research Triangle Park, NC) between the termination of exposure and spleen cell assay." This is a good example of the importance of personal experience in critical analysis of available published data on biological effects of electromagnetic radiation.

A similar study on rabbits exposed for up to 3 months to weak 2450-MHz MW fields was also reported by Chou et al. (47). Two groups of 16 rabbits were exposed in two experiments of 90 days each at 0.5 and 5 mW/cm^2, respectively. The exposure waspreceded by a 2-week adaptation of the animals to the miniature anechoic chambers used for irradiation. Thermographic analysis during MW exposure showed that the SAR ranged from 5.5 mW/g in the head to 7 mW/g in the back at 5 mW/cm^2. Monthly blood samples were taken for hematologic and immunologic examinations, including lymphocyte blast transformation after stimulation with phytohemagglutinin. No significant changes in peripheral blood or in blast-

forming activity of blood lymphocytes were noted during the period of observation. Rabbits are rarely used for immunologic studies because they have relatively labile immune systems and it is difficult to evaluate their immune status. Thus the data do not say much about small changes in the function of the immune system.

Recently Guy et al. (48) reported on a 2-year study involving exposure of rats to 2450-MHz MW fields at 0.48 mW/cm^2 (SAR 0.15–0.4 mW/g). Periodically they collected peripheral blood for basic hematologic and immunologic examinations, but were not able to find any consistent changes that might be related to irradiation.

In summary, studies of long-term irradiation of animals in low-level (below 10 mW/cm^2 for 2450-MHz MWs, SAR below 4–6 mW/g) MW/RF fields do not provide convincing evidence for specific response of the immune system to nonionizing radiation. However, slight and transient immunosuppression, explainable in terms of a chronic nonspecific stress reaction, not related directly to interaction with MW/RF energy, is very possible. There is a lack of fully convincing reports on the immune status itself, as opposed to selected parameters, in animals exposed chronically to MW/RF fields of different frequencies and pulse modulations. It is worthwhile to remember the earlier discussed findings of Adey and his group concerning cellular alterations in cultured lymphocytes *in vitro* caused by exposure to sinusoidal pulse-modulated 450-MHz fields, and the phenomenon of windowing for these alterations. There are no experiments *in vivo* involving exposure of animals to low-frequency modulated MWs with examination of the immune functions. On the other hand, as discussed below, both the higher susceptibility of animals to chronically exposed bacterial and viral diseases, and the data on acceleration of development of neoplasms in mice exposed for months in non-thermal MW fields (the two phenomena that might result from suppression of immune functions in chronically exposed subjects) emphasize the problem of the response to long-term low-level irradiation in MW/RF fields, and they call for further investigation.

INTEGRATED EVALUATION OF IMMUNITY IN MW/RF-EXPOSED ANIMALS

By “integrated evaluation of immunity” we mean an evaluation of the actual immune status of the host, including its nonspecific and specific mechanisms of resistance and reactivity to typical stimulants of immunity (pathogens, transplants, etc.). As mentioned before, there exists no single test or battery of tests that allows precise measurement of the immune status of the organism. The best procedure is to observe the effects caused by factors known to influence immunologic responses. A typical example of integrated evaluation of immunity is the immunization of the host with an antigen followed by the measurement of the level of circulating antibodies against the antigen. Synthesis of antibodies in the organism requires proper function of different immunocompetent cells, including macrophages, T lymphocytes, B lymphocytes, and plasma cells, as well as numerous control, helper and suppressor mechanisms exerted both by cellular and humoral factors. Although we can observe only the final effect (titer of antibodies), we can reach conclusions about the effectiveness of the whole system of humoral

immunity, and concentrate later on an explanation of the function of single elements. Other examples of integrated evaluation of immunity are reaction of animals to transplants, including implantation of neoplastic cells, susceptibility to experimental bacterial and viral infections, and tolerance of factors known to suppress or stimulate immunity (e.g., ionizing radiation and endotoxins).

Surprisingly, in the literature there are only a few investigations where the above models were applied and tested in animals with long-term exposure to electromagnetic radiation. Thus, we still do not know whether long-term irradiation in MW/RF fields results in immunosuppression, or what the biological significance of the possible suppression would be for the host.

For years we have tested susceptibility to experimental staphylococcal and viral (herpes and vaccinia viruses) infections after long-term exposures to 2450 MHz (continuous wave) at different power densities. In an early study (60) we exposed rabbits to 3000-MHz MW fields for 6 hours daily for 6 or 12 weeks at 3 mW/cm^2 (SAR not determined). After the last exposure the rabbits were infected intravenously with known (sublethal) doses of virulent *Staphylococcus aureus*. Both MW-exposed and control animals survived the infection, but a decreased production of granulocytes and a weaker response of granulopoiesis to the infection were observed in the MW-exposed animals. Unfortunately, at that time (1972–1973) no anechoic chamber was available and the exposures were performed in an open field with an absorbing screen opposite the horn antenna. Thus, measurements of incident power density during exposure were questionable, and measurement of SAR was not possible at all. We reexamined the experimental model a few years later after well-controlled conditions of irradiation and measurement were provided at the Center for Radiobiology and Radiation Safety in Warsaw, Poland. This time we exposed mice to 2450-MHz MWs at 5 and 15 mW/cm^2 (SAR of 2 and 6 mW/g, respectively) 2 hours daily, and irradiated the animals for 6 or 12 weeks, 6 days weekly (13). After termination of the exposure in some of the animals, evaluations were made of phagocytic ability *in vivo* (clearance of labeled staphylococci from blood after intravenous injection of killed microorganisms) and delayed hypersensitivity reaction to oxazolone. Other mice (20 per group) were infected with a lethal dose of virulent *S. aureus* (a strain pathogenic for mice). The dose of staphylococci was titered to provide about 60% survival rate in healthy, unexposed mice, to facilitate possible observation of both elevation and fall of the survival rate. The results are summarized in Figure 2.

Exposure of mice at 5 mW/cm^2 (SAR 2 mW/g) during 12 weeks did not result in detectable differences in phagocytic activity, hypersensitivity to oxazolone, or survival of experimental staphylococcal infections compared with sham-irradiated controls (Figure 2). In mice exposed for 6 weeks, improved phagocytic ability and reactivity to oxazolone were observed (Figure 2). At 15 mW/cm^2 (SAR of 6 mW/g), no detectable increase of rectal temperature after termination of the 2-hour exposure session, 12-weeks exposure resulted in significant lowering of the survival rate and phagocytic ability *in vivo* (Figure 2). During further observation (unpublished

results) we found that these phenomena were transient and returned to normal values 1 month after termination of the 12-week exposures. In summary, the results indicate that long-term exposure of mice may result in a lowering of antibacterial resistance with weaker phagocytic ability *in vivo*, and the threshold for these phenomena for mice exposed to 2450-MHz MWs is 5–15 mW/cm^2.

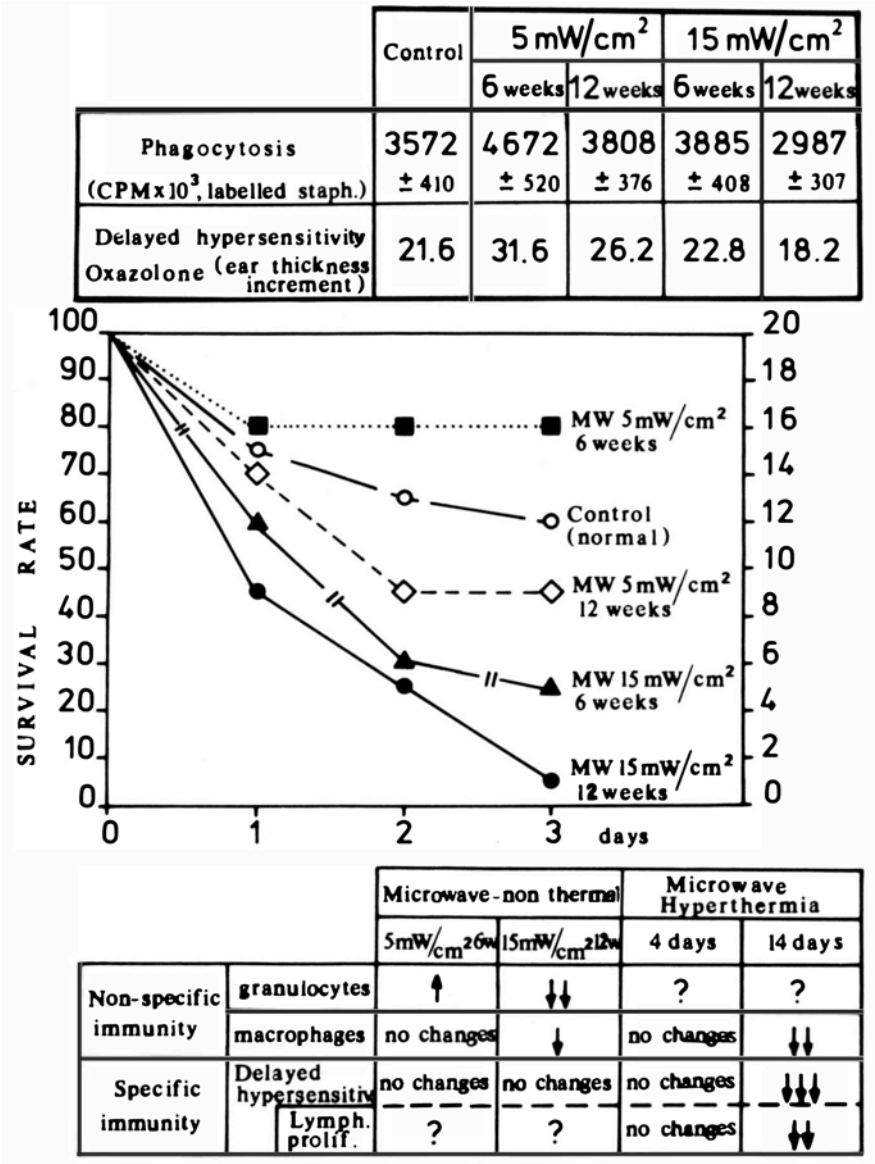

Figure 2. Summary of immunological effects observed in mice exposed during 6 or 12 weeks to 2450-MHz microwaves at 5 or 15 mW/cm^2 (2 hours daily). Note stimulation of phagocytosis after 6 weeks at 5 mW/cm2, depression after 12 weeks at 15 mW/cm^2 (top table), lower survival rate of acute staphylococcal infections in mice exposed to 15 mW/cm^2 during 6 and 12 weeks (graph), and differences in immune response in mice exposed to 5 or 15 mW/cm^2 and in those treated with hyperthermia (bottom table) (4).

SUMMARY OF IMMUNOLOGIC RESPONSE TO MW/RF RADIATION

From the numerous publications on immunologic effects observed in animals exposed to MW and RF fields we have chosen and discussed those that in our opinion, based on many years experience, report experiments performed under acceptable conditions of exposure. We have not discussed the observations of medical and epidemiological investigations of human subjects exposed occupationally to MW and RF radiations. No conclusive results concerning evaluation of the immune status and possible health hazards related to immunologic and/or hematologic findings are possible, especially considering that in the epidemiologic observations only the peripheral blood was analyzed, without more specific tests. There are no screening tests for evaluation of the immune status that may be used in large populations of observed subjects. A recent review of human studies related to occupational MW/RF exposure (61) also does not discuss the immunologic responses.

An overview of the available literature and of our own findings suggests the existence of a biphasic reaction of the immune system to MW/RF radiations—stimulation of the whole system (mainly of humoral immunity) after a single or a few days exposure, followed by gradual, but transient, suppression of the whole immunity with prolongation of the exposure period (up to several months) and/or increasing power density of the fields. Stimulation and suppression of immunity in MW/RF-exposed animals both seem to be transient and inconsistent phenomena. At low power densities the system recovers soon after the exposure. Thermal effects and the concomitant stress situation also stimulate numerous immune functions and should be viewed as a beneficial factor with potential therapeutic applications. There is some experimental evidence that whole-body MW hyperthermia may be beneficial in the treatment of viral infections.

CANCER-RELATED ASPECTS OF EXPOSURE TO LOW-LEVEL MICROWAVE FIELDS

Carcinogenesis has recently come to be viewed as a multi-stage process involving both cellular phenomena leading to neoplastic transformation and uncontrolled growth, and the host's antineoplastic systems including immune system surveillance mechanisms, non-specific cytotoxicity (NK cell activity), and interferon production (Figure 3).

The process of cancer development can be divided into three major stages: initiation, which is the occurrence in the cell nucleus of the decoding of oncogenes as a result of the action of an endogenous or exogenous factor, leading to formation of transformed cells; promotion, which is a selective survival of the transformed cells due to factors acting directly on cellular metabolism or membrane function (e.g., phorbol esters) or to factors influencing the host's antineoplastic resistance (e.g. immunosuppressive drugs, stress); and co-carcinogenesis, defined as mechanisms facilitating formation of neoplastic tumors, including their vascularization and spread (Figure 3).

Environmental factors, including MW and RF radiation, may potentially influence the process of carcinogenesis at various steps, either directly (carcinogenic effect) or indirectly, by

triggering adaptation mechanisms that in turn may influence the natural antineoplastic resistance of the irradiated host. Potential carcinogenicity has been periodically discussed in relation to MW/RF exposure since 1953, when McLaughlin (62) listed various forms of leukemias as one of the possible effects of occupational exposures to radar. More recently, similar suggestions have appeared in the report by Lester and Moore (63), who found significantly higher rate of incidence of cancer mortality in U.S. counties with Air Force bases, compared with counties without an Air Force base. They related the observed differences to prolonged environmental exposure to MW/RF radiation from radars operating at Air Force bases, However, the above data and suggestions have not been widely accepted, and were criticized after reevaluation (64). Nevertheless, the authors still support their original opinion on the relation of cancer mortality to radar radiation (65).

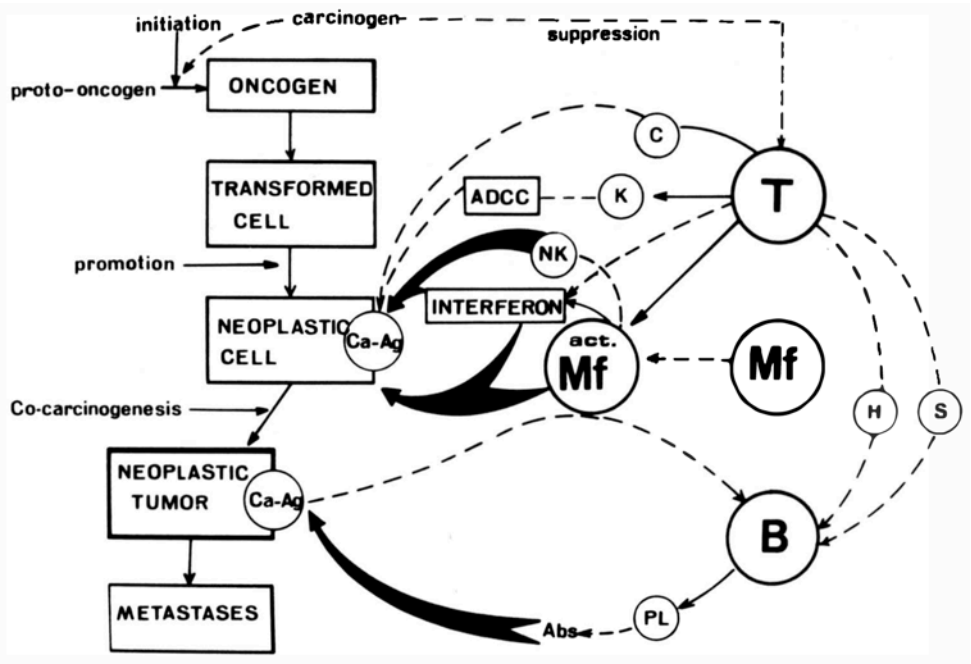

Figure 3. Simplified scheme of cancer development and the influence of immune system on carcinogenesis. A three-stage process of cancer development (initiation, promotion, co-carcinogenesis) is influenced by non-specific and specific cell-mediated and humoral reactions that may cope with small number of neoplastic cells and destroy them. Macrophages (Mf), activated macrophages (act.Mf), T and B lymphocytes (T, B), as well as helper (H) and suppressor (S) T lymphocytes cooperate in these reactions. Neoplastic cells can be destroyed by cellular (NK cytotoxicity) and humoral (interferon, antibodies, Abs) mechanisms.

In a recent review on possible links between RF radiation and carcinogenesis, Kirk (66) stated that the question of RF–carcinogenesis or life shortening remained open, because none of the reports in the literature presented a convincing case for the existence of a significantly higher

risk of cancer induction or life shortening in exposed populations. The situation has however changed in the last few years. Publications from our group (49,50) on accelerated development and growth of benzopyrene-induced skin cancer in mice exposed for months to non-thermal power densities of 2450-MHz MWs (10 mW/cm^2), indicated the tumor-promoting (but not carcinogenic) activity of this radiation, and prompted a few groups of investigators in American laboratories to undertake experimental studies in this field. Most of these studies are still in progress, but evidence of co-carcinogenic properties of MWs *in vitro* (a strong synergy between cancer-promoting phorbol esters and non-thermal MWs in increasing the cell transformation rates) was recently reported (67).

In view of the renewed interest in cancer-related aspects of MW/RF radiation, the recently available information, and the expected progress in this field, it seems desirable to summarize the actual state of knowledge and to stress the further needs and perspectives. Our group presently has several projects on cancer-related aspects of MWs in progress, some of them completed but not yet published, and we will discuss part of this material to give the reader a more complete picture of this intriguing problem.

EXPERIMENTAL OBSERVATIONS

The 1950–1975 literature (68) primarily involves reports of anecdotal and scientifically unsupported cases of neoplasms in workers exposed occupationally to MW/RFs. It does not support the view that these radiations may result in direct or indirect carcinogenic effects, despite the single experimental study of Prausnitz and Susskind (69), who reported an increased frequency of leukosis in mice exposed for 59 weeks to 9270 MHz at 10 mW/cm^2 (thermogenic field) for 4.5 minutes daily (5 days/week). The authors did not specify the type of leukosis, and did not observe the animals after termination of exposures. Considering the conditions of exposure, it seems likely that the observed leukosis was due to heat, which is a known stressor.

Several experimental reports dealing with the effects of MW/RF radiation on development of transplantable, spontaneous, or chemically-induced neoplasms in mice and rats were published during 1975–1985. Preskorn et al. (70) reported that development of tumors following injection of sarcoma cells into 16-day old CFW mice was delayed if the mice were exposed to MWs before birth (*in utero*) on days 11 through 14 of gestation (2450 MHz, 20 minutes daily, SAR of 35 mW/g, thermogenic field). The observation was never confirmed, and thus is difficult to evaluate. Nevertheless, it seems that the slower growth of the sarcoma resulted from stimulation or faster maturation of certain immune functions related to natural antineoplastic resistance (e.g., NK cell activity) in newborn mice after their exposure to MWs during organogenesis. This suggestion is to some degree supported by experiments reported by Smialowicz et al. (28), who exposed rats on day 6 of gestation through 41 days of age (*in utero* and postnatally) to 2450-MHz MWs at 5 mW/cm^2 (SAR 1–5 mW/g) and found that the exposed young rats had lymphocytes that responded to a significantly greater extent to T- and B-lymphocyte mitogens *in vitro*. A similar increase in lymphocyte responsiveness *in vitro* was

observed by the same authors in young rats exposed pre- and post-natally (as above) to 425-MHz MWs (SAR, 3–7 mW/g) (71).

In experiments performed in our laboratories and reported in 1980–1982 (49,50,72) we demonstrated that daily (2 hour) exposures of BALB/c mice to 2450-MHz MWs at 5 or 15 mW/cm^2 (SARs of 2–3 and 6–8 mW/g, respectively) for 3–6 months, resulted in accelerated appearance and growth of skin neoplasms induced by benzopyrene (Figure 4), suggesting a tumor-promoting activity related to long-term exposure to low-level MW fields. Interestingly, the stress from confinement of unexposed mice used as positive controls gave a similar acceleration of skin tumor growth as was observed following exposure at 5 mW/cm^2. Exposures at 15 mW/cm^2 resulted in faster appearance and development of tumors compared with both 5 mW/cm2 and with the controls (49). Exposure of mice to 2450 MHz at the same power densities for 1–3 months resulted in lowering of the natural antineoplastic resistance of the animals, as measured by the number of lung nodules (neoplastic colonies) formed after intravenous injection of a titered number of viable sarcoma cells (Figure 5). Mechanisms of the natural antineoplastic resistance (Figure 3) can cope with a certain number of implanted (or spontaneously developing) neoplastic cells and destroy them without further consequences for the organism. However, the capacity of the antineoplastic resistance is limited, and if a larger number of neoplastic cells is implanted, the lung nodules (colonies growing from single cells) appear. For BALB/c mice, intravenous injection of 2×10^5 sarcoma L-1 cells results in 1–3 visible colonies on the lung surface in 14 days; 1×10^5 cells do not lead to formation of colonies, while more than 5×10^5 sarcoma cells result in the appearance of numerous nodules. When the number of injected sarcoma cells is fixed (e.g., 2×10^5 cells, as in our experiments), an increase in the number of lung nodules is considered as being due to suppression of natural antineoplastic resistance of the organism (Figure 5). The results obtained after exposure of mice to 2450 MHz for 1–3 months (Figure 5) clearly indicated that irradiation at 15 mW/cm^2 (SAR of 6–8 mW/g) resulted in a significantly increased number of lung nodules. The increase was also significant at 5 mW/cm^2 compared with control animals, but the same effect was found following confinement (over-crowding) of unexposed mice (Oc at Figure 5), a known stressor for mice and used here as a positive control.

Although long-term exposure of mice to 5 mW/cm^2 and 15 mW/cm^2 2450-MHz MWs resulted in acceleration of the appearance and growth of two totally different neoplasms (benzopyrene-induced skin cancer and spontaneous mammary tumors in C_3H/HeA mice), and in the lowering of natural antineoplastic resistance of mice, it cannot be said with any confidence whether the effects are related to a direct action of MWs at the cellular or subcellular levels, a non-specific stress reaction, or to general effects on immune response. We subsequently performed further experiments (not yet published) involving possible mechanisms leading to accelerated development of neoplasms in animals exposed for a long time to low-level MW fields. Because these experiments take much time (a few months of daily exposures, several weeks between application of the carcinogen and the appearance of a chemically-induced tumor), we decided to expose mice only at 10 mW/cm^2 (SAR of 4–5 mW/g). This allowed an

increased number of mice in each experimental group. In all experiments the mice were exposed to 2450 MHz continuous wave in an environmentally controlled anechoic chamber for 2 hours daily (5–6 days weekly).

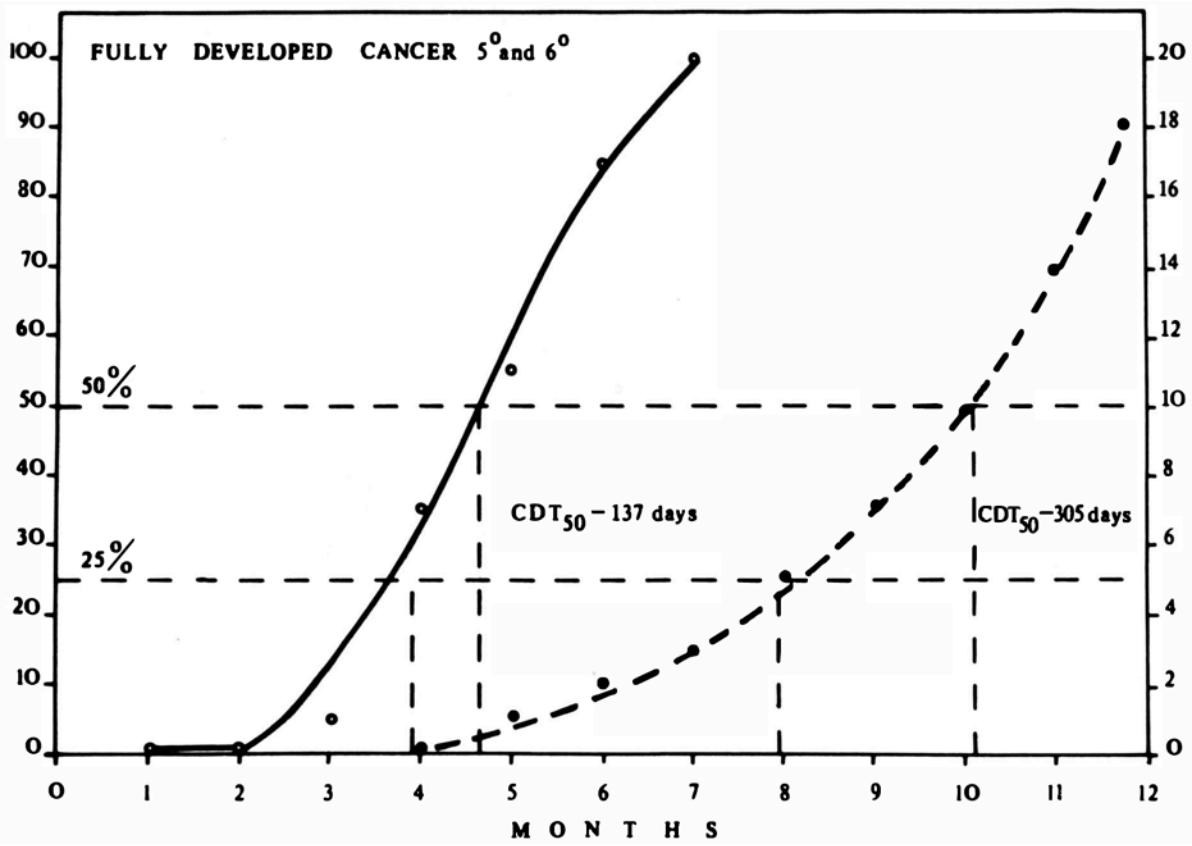

Figure 4. Growth curves of 3,4-benzo-alpha-pyrene (BP)-induced skin tumor in mice exposed daily (2 hours) to 2450-MHz radiation at 10 mW/cm^2 (SAR of 4 mW/g) for a whole period of tumor growth. Note earlier appearance and faster growth of tumors in MW-exposed mice compared with sham-irradiated control. MW-exposed, continuous line; control, dotted line; CDT_{50}, cancer development time in 50% of animals.

We confirmed acceleration of tumor development in MW-exposed mice after application of two other known carcinogens—DENA (di-ethyl-nitrosoamine) and methylcholantrene. DENA, when injected intraperitoneally in mice at 10 or 50 mg/ kg (single dose), results in the appearance of hepatic tumors after 2–6 months in about 80% of the treated animals. However, this carcinogen is also hepatotoxic and, depending upon dose, it also results in necrosis of hepatocyte. During regeneration of liver tissues, when hepatocytes that survived the toxic shock start to proliferate (3–7 days after injection of DENA), the carcinogenic activity of DENA and the neoplastic transformation of the hepatocytes begins. Thus, in this experiment we studied

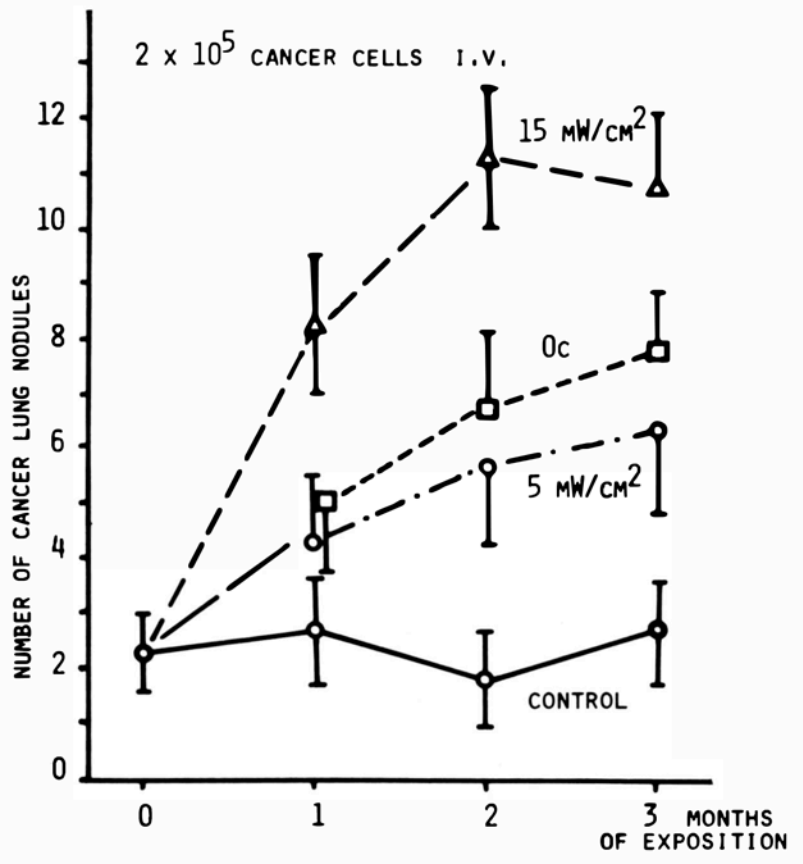

Figure 5. Number of lung tumors (following intravenous injection of 2×10^5 viable sarcoma cells) in mice exposed during 1, 2 or 3 months to 2450-MHz microwaves (2 hours daily) at 5 or 15 mW/cm^2. Oc, mice treated with nonspecific stress of overcrowding (confinement in cages, without exposure to microwaves), as positive controls; control, sham-irradiated mice (49).

the survival of DENA-injected MW-exposed and sham-irradiated mice. After autopsy of each cadaver, histologic examination of the liver in search of neoplastic cells was performed. A significantly shorter survival time and earlier appearance of hepatic neoplasms were observed in the exposed animals. A similar acceleration of tumor growth and appearance was noted after administration of methylcholantrene and exposure to the radiation. Mice were injected subcutaneously with a single dose of this carcinogen , leading to development of sarcomas in 3–4 months. In groups of animals exposed 2 hours daily from the day of administration of methylcholantrene the sarcomas appeared in about 2 months, and grew faster. In both experiments we did not treat animals with any known tumor promoters (e.g. phorbol esters). The animals were exposed only to the carcinogen and/or MW radiation. Since we have never observed development of spontaneous neoplasms in mice exposed for a few months in MW fields (in strains not predisposed for spontaneous cancer), and since this phenomenon is not reported in the literature, we conclude that MWs are not carcinogenic, but that they may promote

(or enhance the activity of other promoting factors) an already initiated process of carcinogenesis.

In another experiment we tested the reaction of mice to subcarcinogenic doses of 3,4-benzo-alpha-pyrene (BP). In general, the scheme of experiments was similar to that used in our earlier investigations (49). Mice were depilated and the skin on the back was pain ted every day with 0.01 ml of BP dissolved in a benzene-methanol mixture. Prior to painting, the mice were exposed for 2 hours to 2450-MHz MWs at 10 mW/cm^2. Mice treated with BP and without MW exposures served as controls. The subcarcinogenic dose of BP (the dose leading to the appearance of skin neoplasms in 10–20% of the animals) was established in earlier trials. A daily dose of 100 µg (0.01 ml of 1% solution of BP) resulted in the development of skin neoplasms in almost all mice in 4–10 months, while 3 and 10 µg (0.01 ml of 0.03% and 0.1% BP, respectively) , applied twice weekly led to the appearance of neoplasms in about 15% of the treated mice. Thus, a subcarcinogenic dose of BP was established as 10 µg twice weekly , and this dose was applied in mice exposed to daily sessions of 2450 MHz MW exposures . It was found that in MW-exposed mice treated with subcarcinogenic doses of BP, 40–50% of animals exhibited skin neoplasms compared with about 15% in those treated with BP alone. The increased frequency of skin neoplasms in MW-exposed mice treated with subcarcinogenic doses of BP was qualitatively different from the earlier discussed acceleration of appearance and growth of neoplasms induced by full carcinogenic doses of BP and other carcinogens. Although it still must be confirmed, the increased frequency of neoplasms after subcarcinogenic doses of BP and exposure of mice to MWs indicates that long-term exposure in non-thermal MW fields may promote development of neoplasms that normally would not reach the clinically detectable stage, independently of the underlying mechanisms.

In another experiment we investigated intracellular levels of cAMP in murine skin epithelium (scraped) from animals treated with daily doses of 100 µg of BP and/or exposed to 2450-MHz MWs at 10 mW/cm^2 for 2 hours daily. The observations were made during one month of exposure and three experimental groups were used: BP alone, BP and MW exposure, and MW exposure alone, with sham-irradiated mice serving as controls. The results are summarized in Figure 6.

Daily exposures resulted in a fast increase of intracellular level of cAMP and in its reactivity with isoproterenol (IPR) (a substance used widely for testing the beta-adregenic response of membrane receptors). The elevated levels of cAMP and the enhanced reactivity with IPR were clearly demonstrable after 14 days of daily exposure· in MW fields (Figure 6). This intriguing observation cannot be explained at present. Levels of cAMP were never before examined in cells or animals exposed to weak MW fields , and there are many known factors that cause an increase of cAMP, including nonspecific stresses. It is accepted that the function of skin is influenced by stress and various reactions from the central nervous system (73) ACTH, adrenal

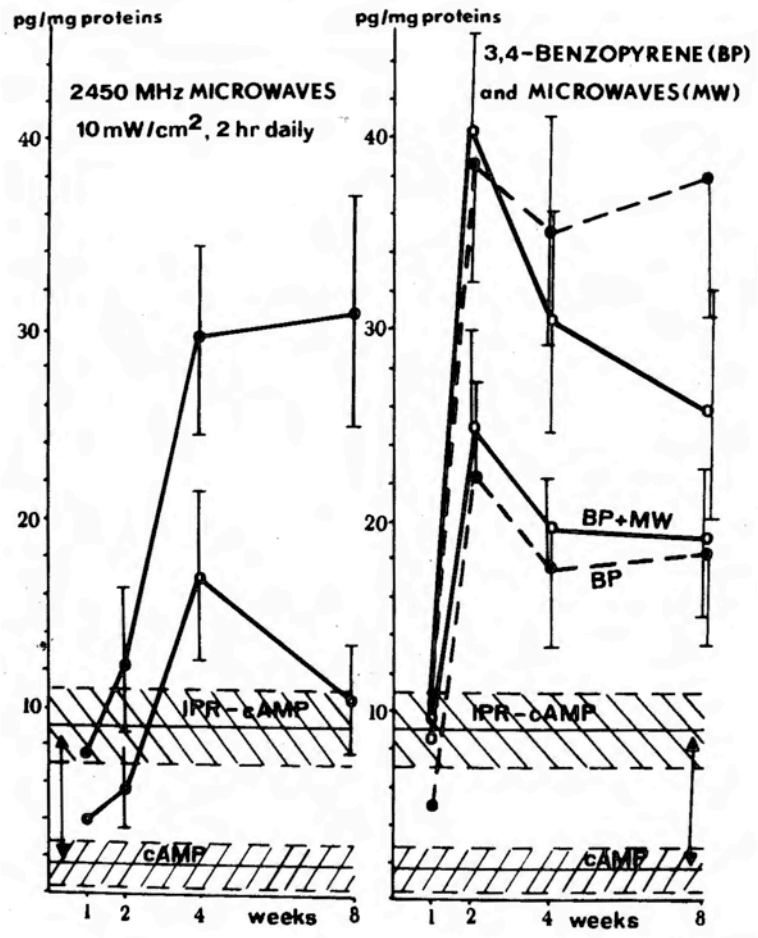

Figure 6. Cyclic AMP (cAMP) (RIA kit, Amersham No. TRK-432) in murine epidermis (scraped) in animals exposed to 2450-MHz microwaves (2 hours daily) for one month (left graph), and in animals exposed to daily doses of 100 µg of 3,4-benzo-alpha-pyrene (BP) and to BP and 2450 MHz microwaves (BP + MW). Basic levels of cAMP are represented by lower curves, with normal levels (in healthy mice) and a standard deviation range presented as a bottom line-filled region. The upper curves and upper line-filled regions represent experimental and control data (with standard deviation) of cAMP levels in epidermis after treatment with isoproterenol (IPR) , a substance in luencing b ta-adrenergic receptors and leading to increase of intracellular cAMP. Note increase of cAMP levels and reactivity to IPR in murine epidermis after exposure of animals to 2450-MHz MWs at 10 mW/cm^2, increase after treatment with BP , however without further elevation in mice treated with BP and exposed to microwaves (BP + MW).

steroids, catecholamines, histamine , and serotonin , released during stress situations, also influence function of skin (74). Thus , the observed increase of cAMP in skin epidermis of mice exposed to MW fields may be due to the concomitant stress reaction. Elevation of intracellular cAMP level in epidermis and enhanced reactivity to IPR were also reported by Murray and Verna (75) for epithelial cells exposed to BP, and by Mufson et al. (76,77) or other carcinogens.

It is believed that the reactivity of the cAMP system is caused by a direct influence of carcinogens and/or promoters on cell growth regulatory mechanisms regulated by the cAMP system (78). Surprisingly, MW exposures that alone caused an increase of intracellular cAMP did not lead to further elevation of this nucleotide when applied to BP-treated mice (Figure 6). Further experiments are needed to explain these observations.

In summary, long-term exposure of mice to 2450-MHz MWs resulted in acceleration of the appearance and growth of tumors initiated by three different carcinogens, and a higher risk of cancer development in animals exposed to subcarcinogenic doses of initiators. The results suggest a tumor-promoting activity of the radiation, but the cause of these phenomena still remains an open question. The experiments were performed only in one animal species and with a single frequency of MWs (2450 MHz). It should be stressed that 2450-MHz MWs is a resonant frequency for mice (maximal absorption of energy), and the situation may be different in larger animals and in human beings. Nothing is known about cancer-related effects of pulse-modulated MW/RF radiation *in vivo*, especially those sinusoidally and amplitude-modulated at 3–100 Hz that, according to Adey and his group, may directly influence cell membranes and cell metabolism.

An increased number of spontaneous malignant tumors in rats exposed f or their life-span to MW fields was recently reported (48,79). This was the largest single study ever made of the long-term effects of MW exposure. Two hundred Sprague-Dawley rats were maintained under pathogen-free conditions and exposed continuously in a unique circularly-polarized waveguide facility to pulsed 2450-MHz MWs (800 pulses per second with a 10-μsec pulse width) at 0.48 mW/cm^2 (SAR of 0.15–0.4 mW/g). Another 200 rats were maintained in sham-exposure waveguides and served as controls. The presence of neoplasms in spontaneously dying animals from both groups was established on the basis of necropsy and histopathologic examinations (about 40 samples from different organs and tissues were taken from each rat). A total of 192 neoplastic lesions (most of them occurring at 19–30 months) were observed , and there were 83 unique combinations of organ and neoplasm-specific diagnoses. The authors divided the observed lesions into benign neoplasms (115 cases), and primary (23) and metastatic (54) malignancies. The frequency of benign neoplasms did not differ significantly between the exposed and unexposed groups (62 cases versus 53 cases), but differences were noted for primary and metastatic malignancies. In the exposed group, 54 malignancies were found (18 primary and 36 metastatic), while in sham-exposed controls only 23 (5 primary and 18 metastatic), the difference being significant at $P < 0.05$. The authors claimed that (79) “the primary tumors occurred earlier in the exposed group than in the sham-exposed,” but they were unable to find a predominance of a specific type of malignancy or tissue of origin. On the basis of neoplastic lesions and diagnoses established for each animal, as listed in the report (79), we tried to reevaluate the frequency of malignancies developing from the hemato-immunologic system (all forms of leukemias and lymphomas, found in different organs). In the MW-exposed animals, 31 cases of hemato-immunologic malignancies were found, while only 19 were found

in the sham-exposed animals; the difference was not significant ($\chi^2 = 3.29$, $P = 0.07$), probably due to the relatively small size of groups in terms of morbidity rate from spontaneous neoplasms (200 rats per group). On the other hand, while listing the primary cause of death of their rats, the authors related only 8% mortality in the exposed group and 3% in sham-irradiated controls to neoplasms (their own reevaluation of the published data). This indicates that most of the diagnosed malignancies were established on the basis of histologic examinations rather than on the presence of clinically detectable disease or tumors. It is well known from analysis of mortality data of large groups of older (above 60 years) human subjects who died from various causes other than malignancies, that careful histopathologic examination frequently reveals (up to 40–60% of subjects) small groups of silent neoplastic cells (most frequently in the thyroid gland) that do not have at that moment any clinical significance, and which probably would never develop in to clinically detectable neoplasms. A similar situation may also occur in older rats , and we therefore believe that when studying the link be tween cancer morbidity and MW/RF fields, only clinically detectable malignances should be taken into account.

The data (79) indicating an increased number of spontaneous malignancies in MW-exposed rats are provocative but far from fully acceptable. The significant difference in frequency of malignancies between the exposed and unexposed group, without differences in any specific type of malignancy or its tissue origin, may be considered only as a trend to increased risk of cancer after prolonged MW exposures. The phenomenon needs further confirmation. For valid conclusions concerning the rate of spontaneous malignancies , the size of the observed groups must be much larger (the order of 1000), and the rates of spontaneous neoplasms in rats must be considered. The data supports the concept of tumor-promoting, but not direct carcinogenic (initiating) activity of MW radiation. In the case of promoting agents the spontaneously-initiated process of cancer development is facilitated, and there are no preferences in organ or type of malignancy. Any group of transformed neoplastic cells that spontaneously appears in any organ or tissue (let us assume even that it appears with the same frequency in MW-exposed and unexposed animals) will be promoted to develop into a detectable neoplasm by MW exposure. Thus, an increased number of malignancies will be found without preference of organ or type.

In summary, the results of studies using different animal models provide only scanty evidence that exposure to low-level MW/RF radiation may in certain cases influence the complicated process of carcinogenesis, with tumor-promoting (direct or indirect) activity possibly being a general phenomenon. The mechanisms leading to these effects are still unknown. The field remains open for good research projects with adequate exposure data, free from possible artifacts.

Another line of search for possible links between weak electromagnetic fields and carcinogenesis is the investigation of cellular/subcellular effects on the process of carcinogenesis *in vitro*. There are presently available valid techniques for neoplastic transformation of cells cultured *in vitro*, including studies on the effect of promoters, and thus it is very surprising that these techniques were applied to bioelectromagnetic studies only very recently. Up to the early

1980s, no laboratory was interested in the influence of MW/RFs on the process of carcinogenesis and promotion *in vitro* un til Balcer-Kubiczek and Harrison (67) reported a strong synergy between phorbol esters (known promoters of carcinogenesis, active *in vitro* and *in vivo*) and non-thermal MW fields in increasing the transformation rates of cells cultured *in vitro* and treated formerly with benzopyrene. In 1981, this group started their investigation on the influence of weak 245 0-MHz MW fields on carcinogenesis *in vitro*. In the first experiments they were unable to find any effect after exposure of cell cultures to MW fields in combination with BP or x-rays without the addition of promoters (80). In later experiments (67), they investigated the carcinogenic activity of 2450-MHz pulsed MWs (120 pulses per second, with an 83 μsec pulse width) combined with BP or x-rays, using an *in vitro* assay for malignant transformation in $C_3H/10T1/2$ line of murine embryonic fibroblasts (established cell line). Experiments were performed at low power densities, not leading to elevation of temperature and corresponding to an SAR of 4.4 mW/g. Additional experiments were performed to assess the effect of a non-cytotoxic and non-transforming concentration of the promoter of carcinogenesis (one of the widely applied phorbol esters, TPA) on induction of transformation in cells exposed to MWs and to x-rays (used as a transforming factor). MWs had no effect on transformation induced by BP or x-rays in the absence of tumor promoter (TPA). On the other hand, treatment of cells previously irradiated with MW and x-rays with TPA (0.1 μg/ml) yielded a statistically significant increase in transformation by a factor of 1.6–3.5 compared with the transformation rates of cells irradiated with x-rays alone and treated with TPA. The authors concluded that 2450-MHz MWs can induce latent transformation damage, which can then be revealed by the action of promoters of carcinogenesis (TPA). The results also suggested that the cell membrane might be a sensitive target for the influence of low-level MW fields. It should be stressed that Balcer-Kubiczek and Harrison applied MW radiation modulated at 120-Hz pulses, which was close to the modulation shown by Adey and his group to exert specific effects on cellular membranes independently of the frequency of the carrier waves. It would be interesting to test the Harrison's experimental model with continuous 2450-MHz MWs at different intensities to estimate whether the increase in neoplastic transformation is related to the carrier wave or to its modulation.

As mentioned before, Byus et al. (9) found strong inhibition of histone protein kinase in cultured human lymphocytes exposed to 450-MHz MWs, sinusoidally modulated at 16 Hz, with a dependence of the effect on the modulation frequency. Recently, the same authors (11) extended their studies in ways that appear directly to relate to cancer promotion. They investigated the effect of cancer-promoting phorbol esters (TPA) on the activity of ornithine decarboxylase (an enzyme present in all nucleated cells, being essential for synthesis of polyamines, which in turn are required for DNA synthesis and cell growth) in cultured hepatoma cells *in vitro* (Reuber H 35 line). Activity of the above decarboxylase was increased 1.5-fold during exposure (1 mW/cm^2), and the elevated activity persisted for several hours after a 1-hour exposure. Also, the increased activity evoked in the cultured cells by TPA was potentiated by prior exposure to the modulated MWs. The authors concluded that the cell membranes were the site of transductive coupling of MW/RF fields modulated at low frequencies, because the tumor

promoter TPA has a specific cell membrane protein kinase receptor (the calcium-phospholipid kinase or protein kinase C) , and the synergy between TPA and the modulated MW fields was consistent with this common site of action (11).

On the basis of Balcer-Kubiczek and Harrison's reports, and the above investigations of his own group, Adey (personal communication) recently offered his own concept and initial model of the cancer-promotion process and its influence by MW/RF fields modulated at low frequencies. The promotion appears to relate to a distorted inward stream of signals from the cell membrane to the nucleus (where carcinogenesis was already initiated by other factors) and to intracellular organelles. MW/RFs modulated at low frequencies may in certain cases (depending upon modulation and time of exposure) act synergistically with the action of promoters, activating the same membrane receptors .

HUMAN STUDIES

Before the suggestions of McLaughlin (62) and Lester and Moore (63), who linked increased risk of leukemia with radar exposures, there appeared two letters to the Editor of the New England Journal of Medicine (81,82) suggesting an association of polycythemia vera and leukemias with occupational exposure to a variety of electromagnetic fields, and three letters to the Editors of Lancet (83-85) with similar observations. A higher incidence of cancer in the electronic industry workers was also postulated by Vagero and Olin (86) on the basis of the Swedish Cancer Environment Registry data. All these reports are based on analysis of death certificates and relation of profession of the leukemia victims with probable exposures to unknown intensities of a variety of electromagnetic fields in electronics, electric engineers, radio and television repairmen, and so forth. None of the above analyses meets the criteria for scientifically valid statistics, nevertheless they stress the need for retrospective and prospective studies resolving the questions raised.

The relevant and acceptable study on delayed health effects in U.S. Navy personnel exposed to radar during the Korean War (87) should be mentioned here. No significant differences were found by Robinette et al. (88) and Silverman (89) between the high and low exposure groups for malignant neoplasms as the cause of hospitalization and/or death (from records of Navy and VA hospitals). However, when three sub-groups of the high-exposure group were developed to provide a gradient of potential exposure, a trend appeared for increased number of malignant neoplasms in the subgroup rated as highly exposed. The weak point of this analysis is the fact that only subjects hospitalized in Navy or VA hospitals were analyzed, and only during a certain period of time (1950–1974). These subjects were only a part of the total population of U.S. military personnel that operated in Korea during 1950–1954, and it is difficult, for example, to evaluate which part of the total population was hospitalized or died during 1950–1974.

Very recently we completed a retrospective study on neoplasm morbidity in Polish military career personnel with relation to present and past occupational exposure to RF/MW fields. Since the obtained results, described in detail in a report of limited distribution (90), will be published

only in part and with a certain delay, we consider discussion of our data to be desirable here. The total population of career servicemen was analyzed, and a subgroup of personnel exposed occupationally to MW/RF radiation (on the b sis of service records) was developed (Figure 7); the E (exposed) group counted about 3% of total population (Figure 8), the rest (97%) was considered as subjects without exposure to MW/RFs (the NE group).

Aim of analysis :	MORBIDITY RATE OF NEOPLASMS (1971-80)
Type of analysis :	RETROSPECTIVE
Populations :	MILITARY PERSONNEL (100 %)
Subpopulations :	EXPOSED (MW/RF) (3%) NONEXPOSED (MW/ RF) (97%)
Data collections :	NEW CASES OF NEOPLASMS (yearly 1971-80) CUMULATIVE YEARLY MORBIDITY RATE (per 10^5/year)
Subgroups analyzed :	AGE : 20-29; 30-39; 40-49; 50-59
	PERIOD OF EXPOSURE (MW/RF) <2; 2-5; 5-10; 10-15; >15
	LOCALIZATION OF NEOPLAMS 12 + BLOOD & LYMPHATIC T 6
Stastistical methods :	Chi-square 2×2 R×C contingency tables ANOVA (subgroups) $p < 0,01$ $p < 0,05$

Figure 7. Retrospective epidemiological study of cancer morbidity in personnel exposed occupationally to microwave and radio-frequency (MW/RF) radiations.

The E group was composed of personnel working on production, repair and use of devices emitting MW/RFs, as well as of those engaged in teaching and research with use of MW/RFs. The accuracy of occupational exposure in the E group was determined on the basis of past and current service records, and on medical records which contained the results of periodic examinations that were introduced in Poland in 1968 for all servicemen exposed to MW/RFs. The extent of daily exposure, power density, frequency, and modulation varied with each individual in the E group. In general, exposure to various types of radar radiation predominated, but exposures to extremely low frequency fields were also noted. For MW radiations, a typical exposure was estimated as 4–8 hours daily at power densities below 0.2 mW/cm^2 (“safety zone,” according to rules operating in Poland) with incidental (several minutes) daily exposures at 0.2–1 mW/cm^2. However, mainly in personnel working on production and repair of MW devices, incidents of short-lasting exposures to higher power densities (estimated up to 10–20 mW/cm^2)

were reported. These exposures resulted from defying safety rules and were difficult to evaluate. The high-intensity exposures were more frequent in the 1960s, when the safety rules were not yet strictly enforced, but still occurred in the 1970s, despite awareness of the possible health hazards of MW/RF radiation. Thus, in practice, it was not possible to estimate precisely the intensity of MW/RF exposures for the whole E group, due to the large individual differences. We divided the exposed subjects into 5 classes; below 2 years , 2–5, 5–10, 10–15, and above 15 years (Figure 7).

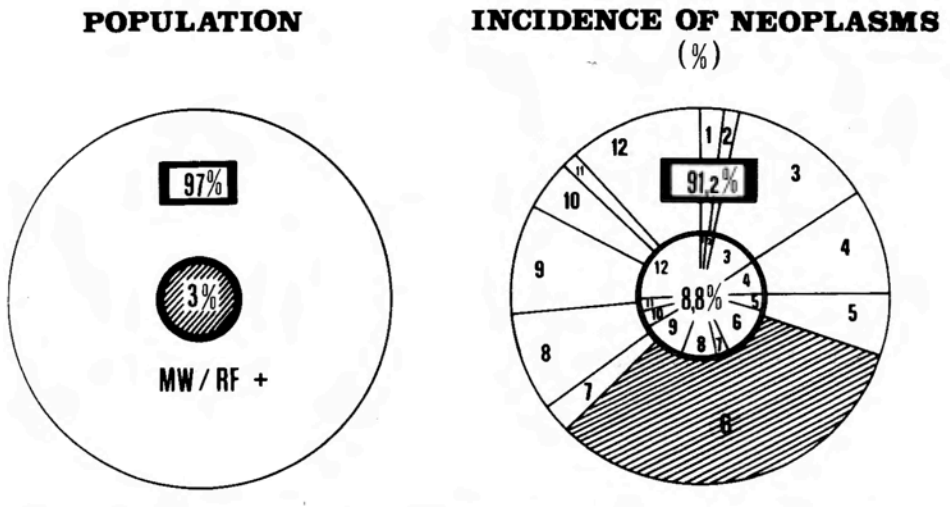

Figure 8. The population and incidence of neoplasms in a retrospective epidemiological study of cancer morbidity in personnel exposed occupationally to microwave and/or radiofrequency (MW/RF+) radiations. Note that the group of personnel exposed to MW/RFs was about 3% of the total population (left circle) and developed 8.8% of all the clinically detectable malignancies found in the total population (right circle). The circle areas marked with numbers 1–12 (right circles) represent frequency of neoplasms in: 1, oral cavity; 2, pharynx and larynx; 3, esophagus, stomach; 4, colo-rectal; 5, liver, pancreas; 6, lungs; 7, bones; 8, skin, including melanoma; 9, kidneys, urogenital tract, prostate; 10, eyes , central nervous system; 11, thyroid gland and other endocrine glands; 12, hematopoietic and lymphatic organs. Note the highest incidence of lung cancer (6) in unexposed group and the highest incidence of hemato-lymphatic malignancies (12) in UW/RF exposed group (right inner circle).

Analysis of cancer morbidity was performed for 1971–1980. All cases of neoplasms that were diagnosed during this decade were registered (on the bas is of data from all department hospitals and medical commissions with care to avoid duplication of cases) and each victim was classified into the E or NE group based on service records and, if possible, an interview. Only subjects aged 20–59 years at the time of diagnosis (with division into four age groups) were considered. Retiring personnel above 60 years of age were not analyzed because their service records were some times doubtful (mainly for those retiring in the early 1970s), and it was not possible to evaluate the occurrence of MW/RF exposures in the far past.

Twelve kinds of neoplasms were differentiated (Figures 8 and 9), and for neoplasms originating in the hemato-lymphatic organs, 6 types of diagnoses were analyzed (Figure 10). From all cases of neoplasms diagnosed during 1971–1980, including their localization and classification in the E or NE group, a cumulative yearly morbidity rate was calculated as: [(total number of neoplasms during 1971–1980)/(mean yearly number of personnel in E or NE group during 1971–1980 × 10)] × 10^5. This gave a morbidity rate of neoplasms expressed as the number of cases per 100,000 subjects per year. The morbidity rate was used for presentation of all results.

The results are summarized in Figures 8–12. In the E group , which was about 3% of the total population , about 8.8% of all the neoplasms appeared (Figure 8). Assuming uniform distribution of neoplasms in the total population, the group E should have accounted for about 3% of all neoplasms (2–4% confidence limits for the size of the population analyzed, $P < 0.05$). This means that the frequency of neoplasms was about 3-fold higher than expected in the E group. The morbidity rate for the NE group was 64.2 cases of various neoplasms per 100,000 per year (at all ages analyzed), while in the E group it was 192.2 cases/100,000/year (Figure 9). Organ localization of the neoplasms (Figures 8 and 9) revealed that the difference between E and NE groups depended mainly on the higher number of neoplasms in esophagus, stomach, colo-rectal region, skin, thyroid gland , and most of all on neoplasms originating from hemato-lymphatic organs. The morbidity rate for all hemato-lymphatic neoplasms was found to be 7.4 cases/100,000/year for the NE group, and 50.8 cases/100,000/year in the E group, the last being about 7 times higher compared with the NE group. Interestingly, no significant difference was found for morbidity from lung cancer, which was the most frequent type of malignancy in the analyzed population. In the NE group (all ages, 20–59 years) the rate for this cancer was 23.6 cases/100,000/year versus 33.2 cases/100,000/year for the E group. However a significantly higher rate for lung cancer was observed in the E group at the ages of 40–49 years. Because hemato-lymphatic malignancies were the most frequent diagnosis in the E group, we analyzed these neoplasms in more detail (Figure 10), It was found that in the E group a higher frequency of lymphatic sarcomas and other lymphomas (but not malignant lymphogranulomatosis), acute lymphoblastic leukemia at a young age, and chronic and acute myelocytic leukemias were found, while the morbidity rate for chronic lymphatic leukemia, as well as f or malignant lymphogranulomatosis did not differ for all age groups, but appeared earlier in the E group (at age 40–49, instead of 50–59) (Figure 10).

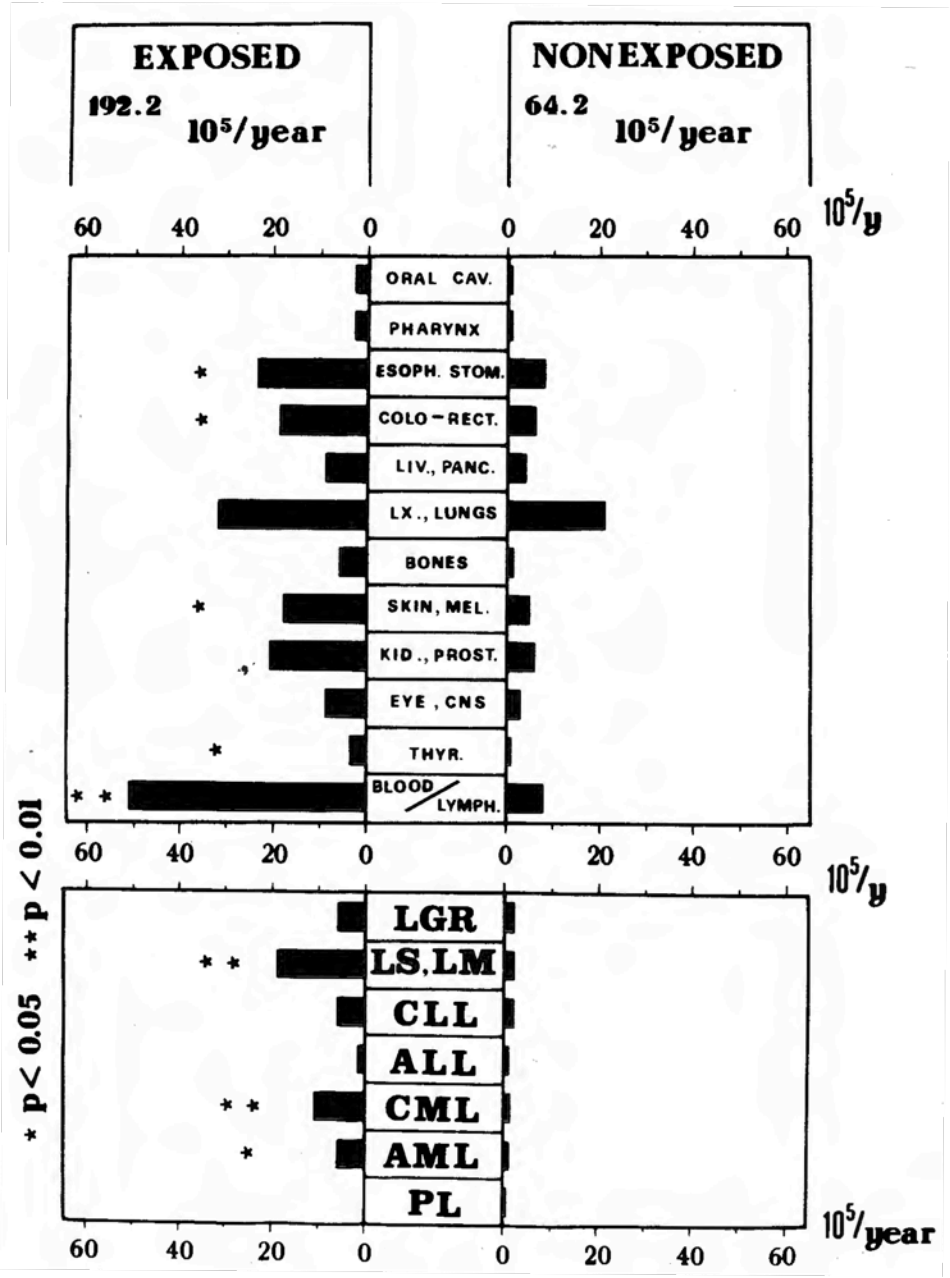

Figure 9. Cumulative yearly morbidity rate of neoplasms during 1971–1980 (expressed as a number of new cases per 100,000 subjects per year) for all ages (20–59 years) in exposed (to MW/RF radiations) and non-exposed personnel. Top histograms, organ localization of malignancies; abbreviations equivalent to numbers 1–12 in Figure 8. Note significant differences in morbidity rate of malignancies in the alimentary tract , skin and malignancies originating from hematopoietic (blood) and lymphatic organs, and no differences in rates for lung cancer (lx, lungs). Bottom histograms, morbidity rate for specific types of malignancies originating from hematopoietic and lymphatic organs: LGR, malignant lymphogranulomatosis; LS, LM, lymphosarcomas and lymphomas; CLL, chronic lymphocytic leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myelocytic leukemia; AML, acute myeloblastic leukemia ; PL, plasmocytoma (plasma cell leukemia).

AGE GROUP	Population TOTAL	Population EXPOSED / NON EXPOSED	LGR	Ly Sa Lymp.	CLL	ALL	CML	AML	PL
20–29	3.6	26.3 / 2.7	18.8 / 2.1	0.3		8.8 / 0.3	8.8		
30–39	3.8	29.7 / 3.0	0.9	0.3	9.9 / 0.3		19.8 / 1.2	0.3	
40–49	11.8	81.3 / 9.9	11.6 / 2.5	46.5 / 4.7	11.6 / 1.4		0.3	11.6 / 1.8	
50–59	32.7	117.6 / 29.6	3.0	58.8 / 8.9	8.9		29.4 / 1.1	29.4 / 5.9	
TOTAL	8.8	50.8 / 7.4	6.0 / 1.8	18.3 / 2.2	6.1 / 1.3	3.0 / 0.1	12.2 / 0.5	6.1 / 1.1	2.2
χ^2		70.01	2.88	29.85	1.11	14.65	45.32	7.52	0.06
P		<0.01	NS	<0.01	NS	<0.01	<0.01	<0.05	NS
RISK FACTOR		6.7		8.3		7.8	9.6	5.5	

Figure 10. Morbidity rate of hematopoietic and lymphatic malignancies (number of cases per 100,000 subjects per year) in personnel exposed occupationally to microwave and radiofrequency radiations and in unexposed controls in different groups of age (20–29, 30–39, 40–49, and 50–59 years). LGR, malignant lympho-granulomatosis; Ly Sa, Lymp., lymphosarcomas and other lymphomas; CLL, chronic lymphocytic leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myelocytic leukemia; AML, acute myeloblastic leukemia; PL, plasmocytoma (plasma cell leukemia). Note the largest differences in the 40–49 age group and earlier appearance of malignancies in the MW/RF-exposed group.

Analysis of the morbidity rates for the four developed age groups (20–29, 30–39, 40–49 and 50–59) showed that the largest difference occurred at 40–49 years. In non-exposed personnel, the frequency of neoplasms (all kinds) did not reach 50 cases/100,000/year, while in the E group a rate of about 350 cases/100,000/year was found (Figure 11). In all other age groups the differences in morbidity were also statistically significant, but not as spectacular as that in the 40–49 group (Figure 11). There was also a high correlation of period of exposure to MW/RF fields with the morbidity rate of neoplasms (Figure 12), and with the coefficient of linear correlation for all cases of malignancies, all age groups, and all five classes of period of exposure ($r = 0.87$). The relation of cancer morbidity rate to period of exposure is best seen in the 40–49 year age group (Figure 12), where there were about 70 cases/100,000/year for those working in MW/RF environment for 2–5 years, about 390 cases/100 ,000/year for those working 5–10 years, and about 450 cases/100,000/year for those working 10–15 years in the fields. A relatively lower morbidity rate in this group (40–49 years) for personnel working above 15 years in the MW/RF environment (about 270 cases/100,000/year) seems to result from two causes. First, most of the

personnel at the age of 40–49 had a 5–15 year period of exposure to MW/RFs, and thus the group with exposure during more than 15 years was relatively small in terms of cancer morbidity rates. Second, at age 40–49, many subjects avoided work with MW/RFs and moved to other duties (command, administration) and, although they were still listed in the E group due to past exposures, the exposures no longer continued.

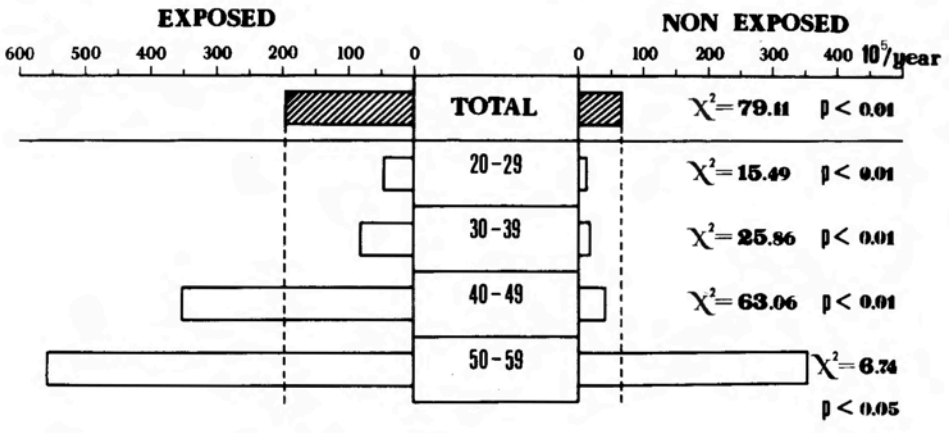

Figure 11. Cancer morbidity rates in exposed (to MW/RF radiations) and non-exposed personnel (all types of malignancies) at various age groups (20–29, 30–39, 40–49, 50–59 years). Note the largest differences at the age group of 40–49 years and statistical significance of differences for all age groups.

In summary , from a retrospective study that covered a large and well-controlled population with a known population of subjects, and that had a relatively long period of observation (1971–1980) the following conclusions may be drawn:

1) The risk of developing clinically detectable neoplastic disease was about 3 times higher for personnel exposed occupationally to MW/RF radiations. The highest risk appeared for malignancies originating from the hemato-lymphatic systems (morbidity about 7 times higher). Other more frequent neoplasms were located in the alimentary tract and in skin (including melanomas) .

2) The highest risk factor of cancer morbidity related to occupational exposure to MW/RFs appeared for subjects at the age of 40–49 who had a 5–15 year period of exposure.

3) Morbidity rates of neoplasms in personnel exposed occupationally to MW/RFs showed strong correlation with the period of exposure.

4) Neoplasms of the same localization and/or type developed earlier (by about 10 years) in personnel exposed occupationally to MW/RFs than in those not working in the MW/RF environment.

AGE GROUP	PERIOD OF EXPOSURE (years)					TOTAL (=EXPOSED)
	below 2	2 – 5	5 –15	10 – 15	above 15	
20 - 29	32.3	58.8				44.2
30 - 39		64.5	96.1	166.7		81.7
40 - 49		71.4	392.9	454.5	272.7	348.8
50 - 59			500.6	611.1	666.7	617.6
Total	32.3	62.5	214.3	247.8	478.3	196.2

$\chi^2 = 40.38$

$p < 0.001$

Coefficient of correlation

$$C = \sqrt{\frac{\chi^2}{\chi^2 + N}} = 0.57$$

Figure 12. Cancer morbidity rates (or all types of malignancies) in personnel exposed to microwave and/or radiofrequency radiations in relation to age groups (20–29, 30–39, 40–49, 50–59) and period of occupational exposure (below 2 years, 2–5, 5–10, 10–15, and above 15 years). The rates represent number of new cases of malignancies per 100,000 subjects per year. Note the highest relation to period of exposure in the 40–49-year group and significance of the whole table (contingency tables RxC) with coefficients of correlation C = 0.57, and Pearson's coefficient of linear correlation r = 0.87 for total data (not shown).

The above findings are intriguing and disturbing for epidemiologists, medical officers, as well as for society as a whole. It must be emphasized that results of retrospective epidemiologic studies are valid only for the population analyzed and the period of observation covered. Further, despite the correlations found and values of correlation coefficients, they do not provide certain evidence of a causative relationship between the effect and the factor investigated. In our population, we assume that exposure of subjects to other harmful and possibly carcinogenic factors, including smoking and drinking habits, in both the exposed and non-exposed groups were similar and we have no evidence to think differently.

At present we cannot offer a convincing explanation for the observed facts of increased risk of cancer in subjects exposed occupationally to MW/RFs, and do not relate this finding directly to interactions of the radiation with the human organism at any level, until the recently started prospective studies of the same population, planned for 1985–1990, is completed. Nevertheless, the available results point to an urgent need for further epidemiological studies, both retrospective and prospective, in well-controlled populations of people exposed occupationally and/or incidentally to a variety of nonionizing radiation, as well as for elucidation of cancer-related problems in experimental investigations.

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