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Study of Nonionizing Microwave Radiation Effects upon the Central Nervous System and Behavior Reactions

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The biologic effects of an electromagnetic field of a frequency of 2375 ± 50 MHz was studied in rats and rabbits in specially constructed absorbant chambers.

The results of the investigations have shown that microwave radiation of 10, 50, 500 $\mu W/cm^2$ for 30 days, 7 hr/day, causes a number of changes in bioelectric brain activity and also in behavioral immunological, and cytochemical reactions. It was found that levels of 10 and 50 $\mu W/cm^2$ stimulate the electric brain activity at the initial stage of irradiation, while a level of 500 $\mu W/cm^2$ causes its suppression, as seen from the increase of slow, high amplitude Δ-waves.

At 500 $\mu W/cm^2$ a decrease in capacity for work, in value of unconditioned feeding stimulus, in investigating activity, electronic irradiation threshold, and in inhibition of cellular and humoral immunity were also observed.

The Soviet side studied the nature of the effect of nonionizing microwave radiation on the biological activity of some structures of the brain, behavioral reactions and also the immunological properties of blood in test animals. This was done in conjunction with the program and agreed to plans for cooperation involving the first stage (in 1976).

Method for Exposing the Animals

Various technical solutions for organizing the experiment are possible (strip-line, parallel plates, waveguide, and others) in studying the nature, degree and repeated effects associated with the effect of electromagnetic energy on an organism. Our experience shows that irradiating animals in the far field of an antenna is an adequate condition for the actual irradiation of a population and consequently most acceptable for research whose objective is the establishment of hygienic norms for environmental factors. This was the method used in our experiments.

In order to decrease reflection of electromagnetic energy from the enclosures, the latter were made from a special absorbing material with a coefficient of reflection not greater than 3% of the incident energy up to a wavelength of 20 cm.

The chamber design used by us for irradiating the animals did not only insure the practical absence of electromagnetic reflection from the walls, but also aided in maintaining the required ventilation and natural lighting (Fig. 1).

The microwave generator (super-high frequency) used for irradiation had a uniformly controllable output of 80 W and operated at a frequency of 2375 ± 50 MHz in a continuous radiation mode. A cylindrical horn 9 cm in diameter with an open angle of the throat (at half power) of 90° and 106° served as the emitter. The distance between the horn and the location of the cage with the animals was 220 cm. The animals were irradiated from overhead.

Four plastic cages with animals were placed into the irradiation zone simultaneously.

Changes in the Bioelectric Activity in the Brains of Animals

The bioelectric activity in the brains of animals was studied on 24 rabbits under conditions of con-

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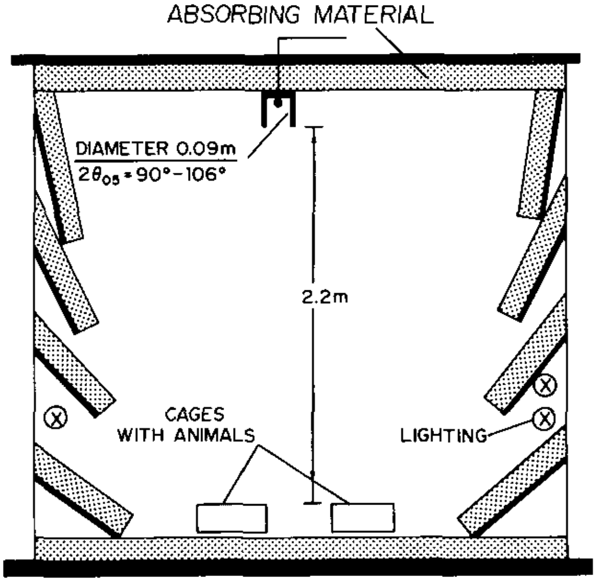

FIGURE 1. Schematic diagram of chamber for animal exposure.

tinuous radiation during the course of 3 months, 7 hr/day at a power density of 10, 50, and 500 $\mu W/cm^2$.

The biopotentials were taken by the monopolar method by use of electrodes which were implanted in the mammillary region of the posterior hypothalamus, the central medial nucleus of the thalamus, and also the sensory and visual zones of the neocortex. The indifferent electrode was attached to the nasal bone. The EEG was registered on an 8 channel encephalograph.

At the beginning of each experiment the initial baseline was recorded by using a functional load, i.e., rhythmic light stimuli at a frequency of 5, 8, and 12 cps. Subsequently, the EEG was recorded after each 10 days during the course of the 3 month effect of superhigh frequency energy. During the recuperative period, the EEG indices were registered every 2 weeks up to complete recuperation. EEG recording lasted 4-50 min in each test.

The obtained data on the bioelectric activity of the brain after total exposure of the rabbits to superhigh frequency energy at power densities of 10, 50, and 500 $\mu W/cm^2$ during a 3-month period showed great variability. The electroencephalographic reaction to the effect of an electromagnetic field did not proceed identically in the subcortical and cortical regions of the various animals.

Nevertheless in statistically processing the data it was possible to determine definite regularities associated with changes in the bioelectric processes taking place in the studied structures. For example, after 1 month of superhigh frequency energy effect at a power density of 10 $\mu W/cm^2$ a reliable ($p < 0.05$) increase was observed on the alpha-rhythm index in the sensory-motor and visual cortex due to the suppression of the slow EEG components. In the case of the subcortical formations (thalamus and hypothalamus) the increase in bioelectric activity developed somewhat later (40 days after initiation of radiation). The total amplitude of the biocurrents increased by 22.5% as compared to the initial value. This type of state of the nervous system of rabbits with periodic increase to the initial background value was maintained throughout the entire period of exposure (3 months).

Increasing the energy of the superhigh frequency field up to 50 $\mu W/cm^2$ has a significant effect on the bioelectric processes. After 2 weeks of radiation the variation in the electrical activity show a more regular nature in the case of the posterior hypothalamus and thalamus for this animal group and the theta-rhythm registers clearly. Its amplitude also increased significantly by 10 to 11 μV on the average, and in some cases by 40 μV.

The use of rhythmic photostimulation at frequencies of 5, 8, and 12 cps showed that the subcortical structures of the brain in the initial state do not master a given light flash rhythm as well as the sensory-motor and visual zone of the cortex. A 2 week period of superhigh frequency energy exposure at a power density of 50 $\mu W/cm^2$ increased the capacity of the neurons to react adequately to discontinuous photostimulation, since unlike the control studies, the rabbits of this test group showed a wider frequency band to which the brain could readjust its own electric oscillation frequency.

After 3 weeks of irradiation, the identity of the biopotentials of the brain was disturbed. Some rabbits showed a greater manifestation of earlier evoked changes in bioelectric activity, others showed some restoration of the initial EEG indices, and still others showed a slowing down of the basic rhythm down to three oscillations per second with some synchronization of processes at that frequency.

After a 30 day effect of super-high frequency energy at a power flux density of 50 $\mu W/cm^2$, the majority of the animals exhibited a gradual increase in the density of slow waves in the 1-3 cps range.

Initial change of the bioelectric activity in the sensory and visual zones was also noticed after 2 weeks of super-high frequency energy effect at a power flux density of 50 μ W/cm^2 (Fig. 2). However, these changes were expressed more intensely in the cortex than in the subcortical structures. The manifestation of the electromagnetic effect in this area took the form of a disturbance of the EEG frequency spectrum, i.e., a verifiable decrease in the number of delta-range oscillations and an increase in the alpha-

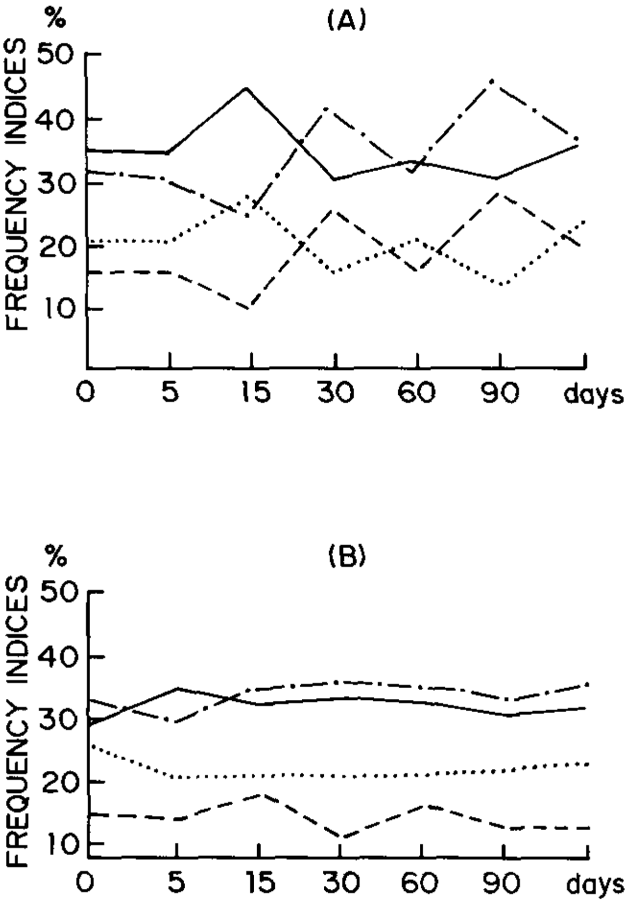

FIGURE 2. Dynamics of the alpha and delta rhythm of (*A*) test rabbits and (*B*) the control group under the effect of super-high frequency energy at a power level of 50 $\mu W/cm^2$: (——) alpha rhythm, sensory motor region; (– –) delta rhythm, sensory motor region; (– · –) alpha rhythm, visual region; (. . .) delta rhythm, visual region.

and beta-range potentials. In the case of the sensory motor region, the delta-rhythm index fell by 6.1% on the average while the alpha-rhythm index rose by 9.2% on the average. In the case of the visual region, the first index fell by 7.7% and the second rose by 9.7% respectively. Under these conditions, no quantitative changes were noticed in the theta range. This obviously attests to the stability of the theta-wave generating mechanism which increases the oscillation frequency more rhythmically as working conditions change. The amplitude is increased by 8.7-10.8 μ V on the average.

In this period the adaptation to the rhythm of flashing light at frequencies of 5, 8, and 12 cps appeared more pronounced than it was initially.

After 1 month and with further exposure to microwaves at 50 $\mu W/cm^2$ there were observed changes in the bioelectrical activity of sensory-motor and optical regions of the cortex. These changes were analogous to the subcortical tissues of the brain with a certain increase in inhibitions by the third month of exposure.

The effect of a microwave field of 500 $\mu W/cm^2$ caused the suppression of bioelectrical activity in the brain which was expressed as an increase in slow delta waves of high amplitude. This occurred after 2 weeks in the regions of the cortex. This effect differed for the exposures to microwave power densities of 10 and 50 $\mu W/cm^2$.

After the end of irradiation (during the recovery period) the different animals had a different EEG measurement. This indicates the different, individual rate of recovery.

The EEG's of control animals did not show significant changes (Fig. 3). As a conclusion one can assume that the effect of low intensity microwaves at a power density of 10 $\mu W/cm^2$ and of 50 $\mu W/cm^2$ stimulate the bioelectrical activity of the brain. This is indicated by the increase in the regularity of the basic theta-rhythm and the degree of similarity of the form of oscillation with a parallel increase of its amplitude in the thalamus and posterior hypothalamus. In the cortex regions, the increase in activity was connected with the raised excitability of the brain. It produced an increase in the specific gravity of high frequencies in the range of alpha- and delta-bands with the background of suppression of low frequency delta-bands.

Also the enhancement of the adaptability to the rhythm of light flashes and the broadening of the

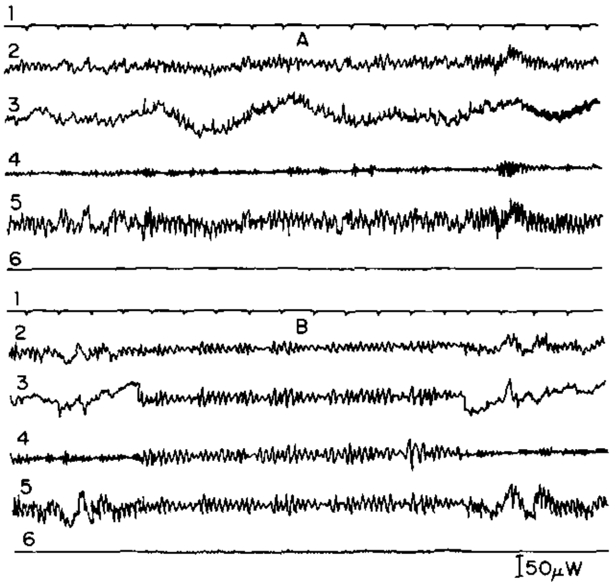

FIGURE 3. EEG of rabbit (*A*) prior to exposure and (*B*) after 1.5 months of exposure to super-high frequency energy at 10 $\mu W/cm^2$: (1) time; (2) EEG of hypothalamus; (3) EEG of thalamus; (4) EEG of sensory motor region; (5) EEG of visual region; (6) stimulus.

span of the given frequencies of the photostimuli, which are absorbed by the brain, indicate an increase in the excitability and instability (functional mobility) of the nervous systems during the investigated exposures.

The subsequent changes in the cortical centers, which manifest themselves shortly after the primary EEG reaction to the effect of super-high frequency energy and which appear in some animals as an intensification of the stimulation of bioelectric processes, in others as in the partial restoration of initial potential, and yet in others as a retardation of the basic EEG rhythm with some synchronization, can be treated as adaptive restructuring of the CNS to the influencing factors.

The gradual increase in the slow high-amplitude activity, after 1 month of irradiation by microwaves at 50 $\mu W/cm^2$ and after 10–15 days of microwave irradiation at 500 $\mu W/cm^2$, is presumed to be related to the development of inhibition in different regions of the brain.

Behavioral Reactions of Animals Exposed to Nonionizing Microwave Radiation

It is now widely recognized that behavioral responses are the most sensitive indices for studying the negative effects of the environment. They reflect the general condition of an organism. However, these methods are used insufficiently in studying the effects of microwave radiation.

In this respect, the most studied subject is the effect of a microwave field of thermal and subthermal intensity (Pd 5 $\mu W/cm^2$ and higher) on conditioned reflex activity. A number of studies have been conducted on the suppression effects on rats of microwave radiation, i.e., the increase in the latent period and decrease in the conditioned reflex level (*1, 2*). The distortion of differentiations (*2*) and decrease in the rate of changes of the learned conditioned reflexes (*3*) were also studied. At the same time, according to other authors, in a number of cases either the changes in the higher nervous activity as effects of the microwave field of subthermal and thermal intensity are not observed at all (*4*) or a stimulation effect is observed (*5*).

There is very little information in the literature about the effect of fields of low intensity on conditioned reflexes. The only available information describes the disruption of development and of preservation of a conditioned reflex (*6, 7*) and also the changes of phases of conditioned reflexes (*8*).

The suppressing effect of a microwave field (of thermal and nonthermal intensity) had been observed in relation to other behavioral responses of the organism: work capacity and investigative activity (*9*), mobility (*7*), and convulsive response to a sound (*10*). On the other hand, there are studies in which authors did not detect changes in locomotor activity caused by exposure to a microwave field (*11*).

A study of the effect of the microwave field of nonthermal intensity on the behavior of animals was undertaken, since the effect of low intensity microwave fields on behavior had not been studied sufficiently and also since changes in behavioral responses could precede or accompany other disturbances.

As indices of this function, parameters were recorded which give the characteristics of the inborn forms of behavior (investigative, feeding behavior, and aggressiveness); conditioned reflex activity (the rate of development of conditioned reflex); effector and receptor behavioral reactions (locomotor activity, working capacity, and skin sensitivity to irritation by electricity).

Search locomotor activity was recorded for 3 min (three times during the first minute) by using the open field test. Animal activity during the first day of recording of indices, at each point, was considered search activity, and the activity recorded on the second day was considered to be locomotor activity.

The latent period of response and its value (amount of consumed food) was recorded as the unconditioned food response parameter.

The degree of aggressiveness was evaluated by the onset of fights between the test and control group rats. The flight was stimulated by electric shocks through the bottom of the animals' cages.

The time during which a rat remained on a rotating treadmill was taken as the measure of the work capacity of the animals under dynamic load. Static load was measured by the time during which the rat remained on an inclined plank.

There were three series of experiments with rats. In the first one the animals were exposed to microwaves at 500 $\mu W/cm^2$ for one month, 7 hr/day. In the second and third series of experiments the animals were chronically exposed to microwaves for 3 months at 50 and 10 $\mu W/cm^2$.

The recording of indices was taken prior to beginning of exposures and also on days 10, 20, 30, 60, and 90 of the experiment.

As a result of these experiments it was determined that the microwave field causes a number of changes in the behavioral responses of an organism.

Thus, the effect of exposure to 500 $\mu W/cm^2$ results in decreasing behavioral search activity and suppression of food response (decrease in the magnitude and increase of the latent period of response)

(Fig. 4). In the case of static and dynamic load tests, work capacity decreased (Fig. 5). Subsequent changes during exposure to super-high frequency energy at a level of 50 and 10 μW/cm² in the search activity of rats were recorded only on the 30th day of the experiment and remained unchanged on further exposure (90 days) (Fig. 6).

The threshold for electric irritation of the skin showed several phases: higher sensitivity of the animals was observed between the 10th and 20th days of exposure and thereafter fell to the level of the control group for those rats which had been exposed to 50 μW/cm² (Fig. 7).

On the 90th day of exposure, the rats were irradiated up to show five conditioned reflexes at one time (Fig. 8). The rats had a significantly lowered reflex execution rate and a reduced number of intersignal reactions. Thus it follows from our data that even exposure of animals to 10 μW/cm² results in disturbance of various forms of inherited behavior and also in changes in the functional state of muscular and receptor apparatus. Such a multifaceted effect of the given super-high frequency exposure level makes it possible to assume the presence under these conditions of a general suppression effect of the radiation on the function of the central nervous system.

Changes in the Immunological and Cytochemical Indices of Blood

In the last few years the problems of the immunological reactivity and the functional state of immunocompetent white blood cells which take part in the immune protection of the organism exposed to the physical factors of the environment have become the subject of substantial interest to scientists. This became possible because achievements in noninfectious immunology, and, especially, because of the discovery of the mechanisms responsible for nonspecific immunological protection and the determination of the roles played by T and B lymphocytes, which compose an integrated system which provides an immune response to the exogenic effects and to the cell homeostatis of the organism.

We investigated in a special series of experiments the effect of a low intensity electromagnetic field in the microwave band (500, 50, and 10 μW/cm²) on the condition of the cell and humoral immunity and the cytochemical indices of blood. In this experiment, 195 white rats having an average weight of 150-200 g were used. They were housed under identical conditions.

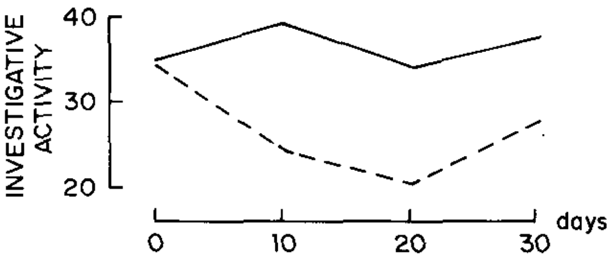

FIGURE 4. Behavioral search activity in rats: (——) control animals; (– –) animals exposed to microwaves at a level of 500 μW/cm².

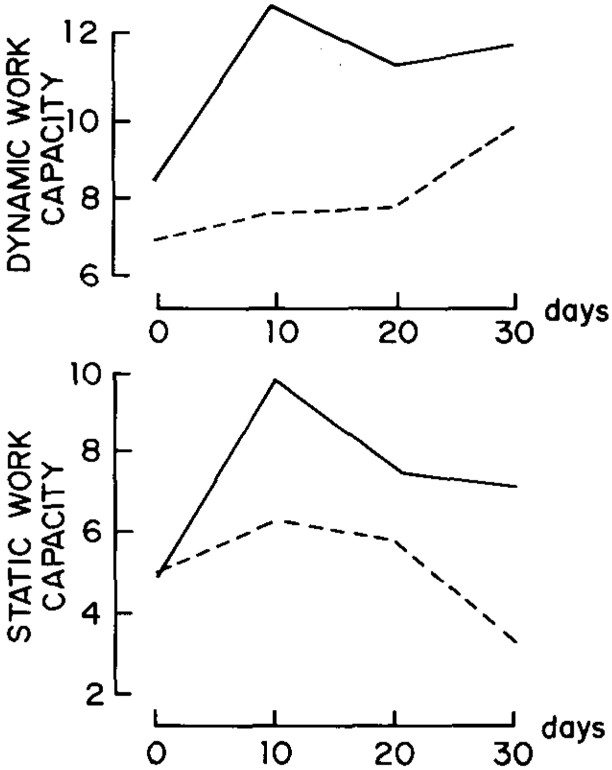

FIGURE 5. Effect of microwave exposure on static and dynamic work capacity: (——) control animals; (– –) animals exposed to microwaves at level of 500 μW/cm².

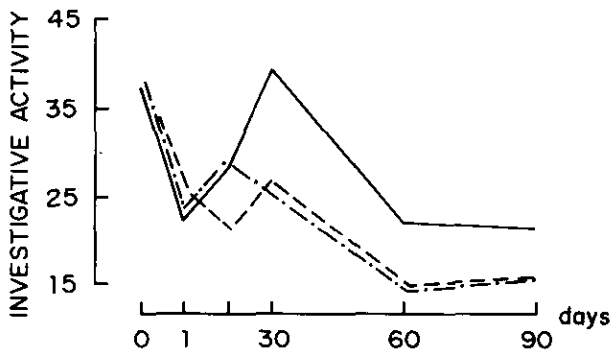

FIGURE 6. Effect of microwave exposure on investigative activity of rats: (——) control animals; (– –) animals exposed to 10 μW/cm²; (– · –) animals exposed to 50 μW/cm².

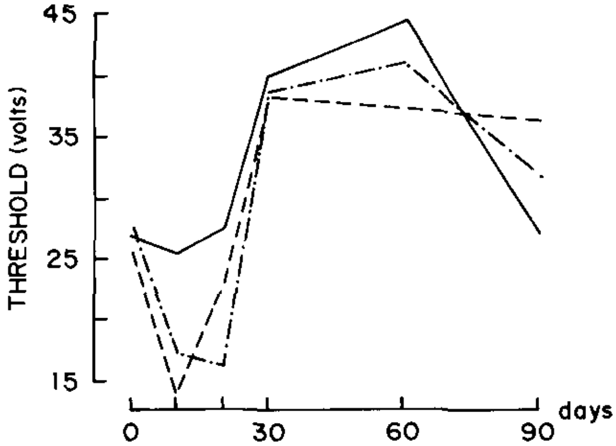

FIGURE 7. Changes in threshold for electric irritation of skin: (——) control animals; (– –) animals exposed to microwaves, 10 μW/cm²; (– · –) animals exposed to microwaves, 50 μW/cm².

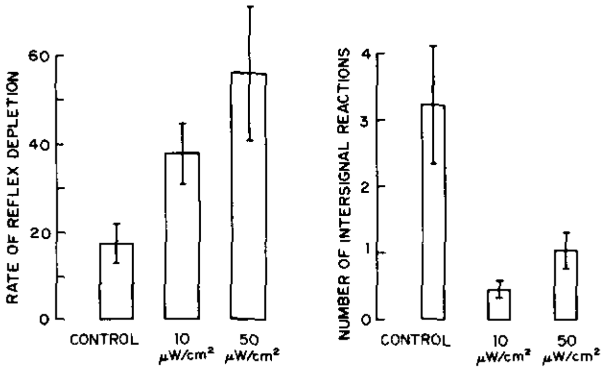

FIGURE 8. Changes in conditioned reflexes as a function of level of microwave exposure.

The investigation of the blast transformation of lymphocytes was done by the methods of Kopelyan and Grigoryeva (*12*). This reaction consisted of recording the number of cells transformed into blast (transitional cells and actual blasts), macrophages, and small lymphocytes. This reaction utilized nonspecific biostimulating agent-phytohemagglutinin and antigen made from the tissues of the brains of exposed animals.

The rosette-forming cells were detected in the spleen and thymus by using the method of Haskill et al. (*13*). Use of this method provided information about the state of the humoral and cellular immunity in general by differentiation of the cell population between T and B lymphocytes.

The completion of the phagocytic reaction was evaluated by the method of Berman and Salvskaya (*14*). Not only was the absorption function of neutrophils considered, but also of their digestive capability.

The glycogen content in neutrophils was determined by using the McManus method (*15*), alkaline phosphatase by the Kaplow method (*16*), and L-glycerophosphate hydrogenase by the method of Hess, Scarpelli, and Pearse (*17*). The evaluation of the obtained data was done by computing the mean cytochemical factor and by a count of positively reacting cells.

These indices were studied under dynamic conditions throughout and after exposure.

Analysis of the obtained data shows that microwave exposure at 500 μW/cm² leads to a sudden and statistically significant disturbance of the immunological system of an organism (Fig. 9). This disturbance is indicated by the substantial suppression of the phagocytic capability of neutrophils and by a reduction of the functional activity of immunocompetent cells which are responsible for cellular and humoral protection reactions. At the power level shown, the microwave exposure leads to a significant lowering of the number of blasts. If the number of transformed cells prior to exposure was 32.7 ± 0.59%, then after exposure, it decreased to 15.6 ± 0.32%. After the end of exposure, the animals retained the steady suppression of the stimuli of lymphocytes by the phytohemagglutinin for 6 months. By the end of the recovery period (after 10-12 weeks) the process of transformation of small lymphocytes into blasts returned to the initial level. During exposure, the number of macrophages kept decreasing at a statistically significant rate, however, already a month after the end of exposure the reactions of macrophages were on the same level as the ones of the controls animals.

A significant decrease in the number of rosette-forming cells in the spleen was observed immediately after exposure (Fig. 10). In thymus, these remain practically on the same level. The repeated statistically significant suppression of the process of forming rosettes is observed in a month after the end of exposure. During the same period there is a significant decrease in the number of cells that form rosettes in the thymus. Comparison of the data of the studied reactions of the observed disturbances in the immunological system of the organism exposed to microwaves at 500 μW/cm² indicates a "directional" nature. The effect of microwaves at this level stimulates the cellular and humoral immunity. The statistically significant increase in the absorption and digestion function of neutrophils and peripheral blood is observed during exposure and immediately after termination. This process is accompanied by an increase in the content of glycogen and of alkaline phosphatase in neutrophilic leukocytes and by the increase in the number of positively reacting cells.

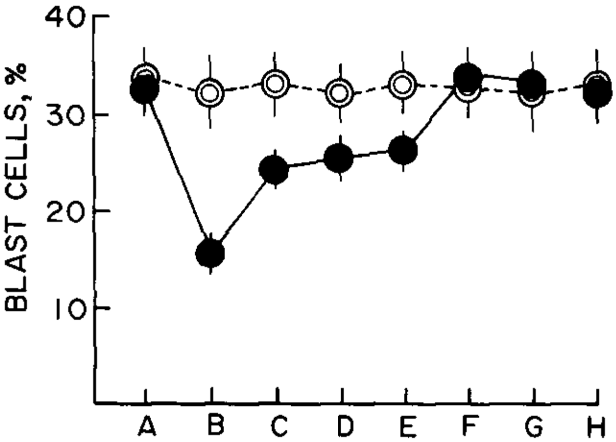

FIGURE 9. Dynamics of the content of blast cells in the peripheral blood of white rats exposed to microwaves for (○) control animals and (●) animals exposed to 500 $\mu W/cm^2$: (*A*) background; (*B*) immediately after exposure; (*C*) 2 weeks after exposure; (*D*) 4 weeks after exposure; (*E*) 6 weeks after exposure; (*F*) 8 weeks after exposure; (*G*) 10 weeks after exposure; (*H*) 12 weeks after exposure.

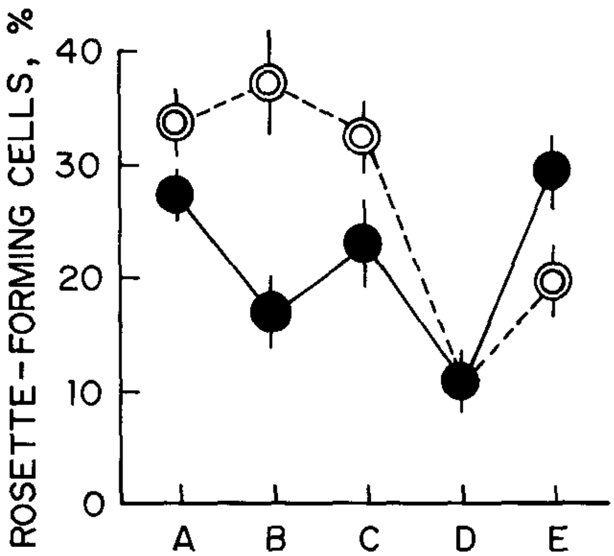

FIGURE 10. Dynamics of immune rosette formation under the effect of microwaves (500 $\mu W/cm^2$) in (○) thymus and (●) spleen: (*A*) background; (*B*) immediately after exposure; (*C*) 2 weeks after exposure; (*D*) 4 weeks after exposure; (*E*) 6 weeks after exposure.

At the same time, stimulation of the lymphocyte reaction of blast transformation and the growth of the number of rosette-forming cells is intensified. This information indicates that the T and B systems of immunity are active. However, continuing research will make it possible to evaluate the functional value of a coordinated interrelation of various factors of cellular and humoral immunity, and the biological significance of a number of other disturbances in the organism. The purpose of this research is to learn about the characteristics and about the degree of recovery of the functional state of the appropriate systems of an organism.

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