MR 1826

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MAIN SUBJECT HEADING:

AN	HU	AT	IH	M
ANALYTICS	HUMAN EFFECTS	ANIMAL TOXICITY	WORKPLACE PRACTICES-ENGINEERING CONTROLS	MISCELLANEOUS

SECONDARY SUBJECT HEADINGS: AN HU AT IH M

Physical/Chemical Properties

Review

Animal Toxicology

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Engineering Controls

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of results. Cell growth response depends on many factors, including the technologic details of thin-layer growth, irradiation techniques, the quantity of radiation reflected, and how much radiation is actually entering the cells. In our hands, several frequencies that Dr. Blackman used had no effect on the cells. Some frequencies produced inhibition of cell growth, although in certain cases, the effect was barely noticeable.

DR. C. C. JOHNSON: In your radiation facility, there was a control dish and beside it a dish wrapped in foil. The edges of the foil appeared to be quite sharp, so the field concentrations might have produced many orders of magnitude greater energy deposition around these edges. Did you confirm that foil placement did not change your exposure conditions?

DR. BLACKMAN: We used the near-field probe described by Swicord to measure the field in which the microwave dish was placed. The power varied no more than 10%, and the same measurement results were obtained with the adjacent dish, covered or uncovered.

DR. K. D. STRAUB: Why did you use 2.45 and 1.7 GHz?

DR. BLACKMAN: We used 2.45 GHz initially, because it was the only frequency available. Dr. Webb suggested that we might find effects at 1.7 GHz.

DR. STRAUB: If the effects you are seeking are due to rotational transitions, at the temperatures at which you work, you would assume that you would have a J–0 to 1 transition, but it is not populated. It is likely that the rotational states exist in higher populations. One would expect that at the rotational frequencies you are describing, the transitions would be in the 2–3, 3–4 transition range. You should try the higher frequencies.

DR. BLACKMAN: We will have systems available to us soon that will allow us to examine higher frequencies.

DR. F. S. BARNES: Did you measure the line widths of the rotational transitions? Only gases have line widths narrow enough to reveal any true resonance. Has anyone seen any line widths that are less than a few gigahertz wide in this range?

UNIDENTIFIED SPEAKER: The answer is no.

MR 1826

EFFECTS OF ELECTROMAGNETIC RADIATION ON IMPLANTATION AND INTRAUTERINE DEVELOPMENT OF THE RAT*

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INTRODUCTION

Effects of ionizing radiation on rapidly growing cell systems, such as bone marrow, malignant tumors, and embryos, are well known.[1] That nonionizing radiation affects intrauterine embryo development was, however, not considered possible until recently.[2–5] It was believed that the quantum energy of nonionizing radiation was much too small to cause any damage. Whereas the quantum energy of diagnostic x-rays for medical purposes is between 25 and 150 keV, that of nonionizing electromagnetic radiation, for example, short waves, with a frequency of 27.12 MHz reaches only 8×10^{-7} eV.

MATERIALS AND METHODS

In 749 rats pregnant with 7800 embryos, we examined the effect of high-frequency treatment of the abdomen on the intrauterine development of the embryo. Rats between Days 1 and 16 of pregnancy were treated only once for a short time with a short wave field (27.12 MHz, i.e., a wavelength of 11.05 m, in air). The high-frequency electromagnetic radiation doses were measured thermally, since a more suitable method was not available. The rectal temperature was continuously monitored with a mercury thermometer for rats. Because the temperatures rose at varying rates, they were measured individually. When the rectal temperature reached a certain level predetermined for each experimental group, the rat was removed from the vhf radiation field. An Ultratherm 603 (Siemens) with a Servomat automatic tuner was used to generate the high-frequency radiation. The electric power was 55, 70, and 100 W in three experimental groups. The coil field electrode Minode[6] served as the applicator. The fetuses were obtained by sectioning on Day 20 of pregnancy. They were weighed and examined for externally visible malformations (visceral and neurocranial and of the eyes, extremities, tail, and palate). To identify spinal and skeletal defects, the alizarin red staining technique was employed. In addition, both embryos found in a state of resorption (abortion after implantation) and corpora lutea graviditatis were counted. By subtracting the number of mature fetuses plus the number of resorbed embryos from the number of corpora lutea graviditatis, the preimplantation loss was calculated.

*Supported by Deutsche Forschungsgemeinschaft Grant Di 199/1.

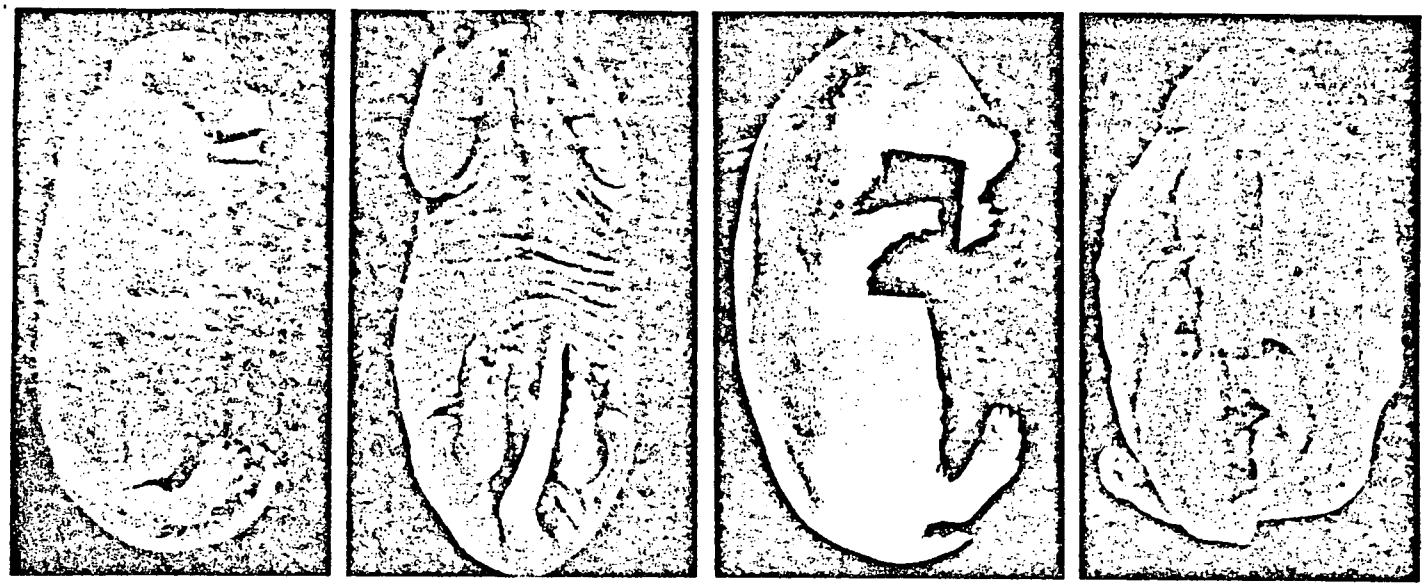

FIGURE 1. Typical malformations, which depend on the pregnancy phase during vhf irradiation treatment. *Left*, several malformations of the neurocranium caused by vhf irradiation on Days 9 and 10 of pregnancy; *left center*, kinked tail caused by vhf irradiation on Days 13 and 14 of pregnancy; *right center*, short tail and hand defects as typical malformations after irradiation on Days 13 and 14 of pregnancy; *right*, cleft palate caused by a vhf irradiation treatment on Day 15 of pregnancy.

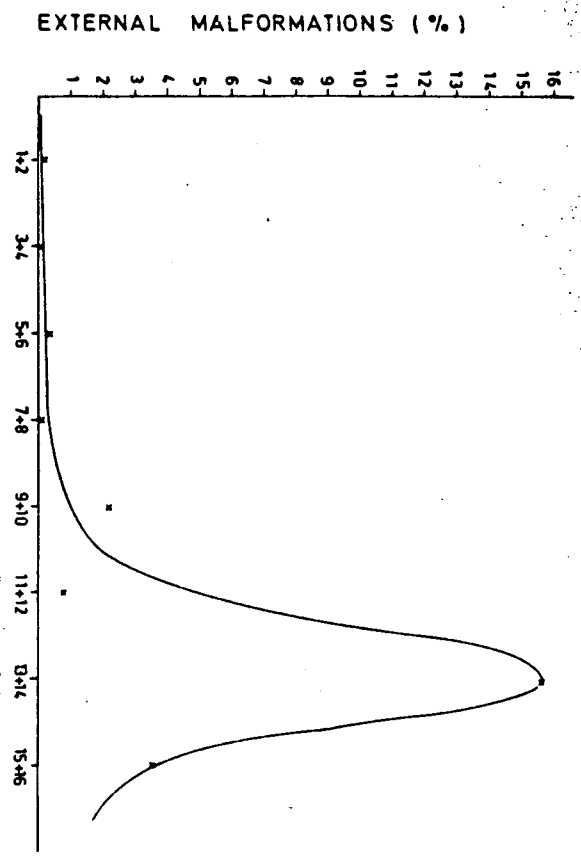

FIGURE 2. Frequency of external malformations, which depends on the treatment day.

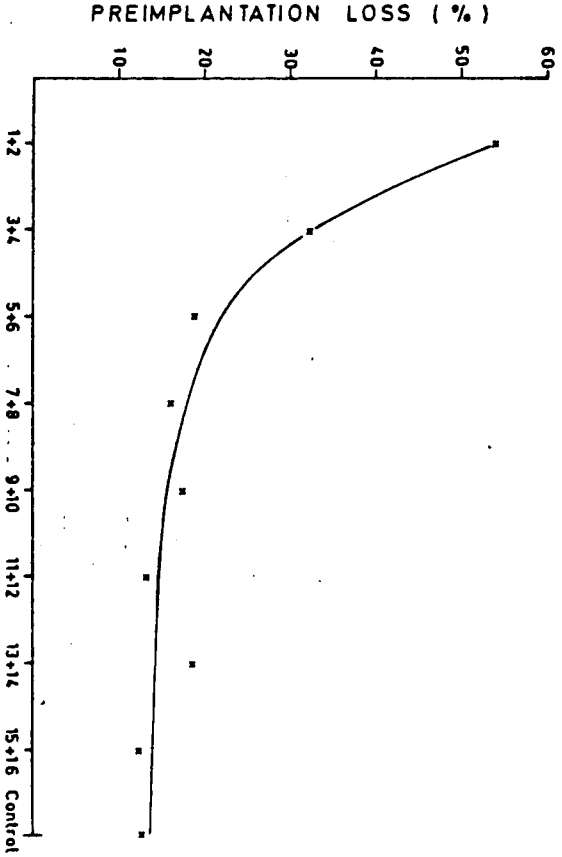

FIGURE 3. Prior to implantation, vhf irradiation kills a high percentage of the blastocysts. In the Figure, only pregnancies in which at least one fetus reached term are included. For the pregnancies in which all the blastocysts were resorbed, the preimplantation loss would be much greater.

RESULTS

Dependence of vhf Effects on Pregnancy Phase

Examination first revealed that high-frequency effects are dependent on the pregnancy phase. We were able to create a spectrum of malformations (FIGURE 1), in which the type corresponded directly to the teratogenetic phase of determination at the treatment time. FIGURE 2 shows that the frequency of external malformations was dependent on the day of treatment.

Examination furthermore proved that an early intensive high-frequency treatment, especially prior to blastocyst implantation, usually results in the death of the embryo (FIGURE 3). Rats in later pregnancy phases, after completion of organogenesis, also are dangerously affected by short wave treatment due to heat accumulation in the amniotic sac (FIGURE 4).

Dependence of vhf Effects on Treatment Intensity

A second very important aspect was that the frequency of malformations and abortions depended on the intensity of vhf radiation treatment. In particular, we found a lower threshold of intensity at which malformations occurred than has been previously reported. FIGURE 5 shows the malformation frequency on Days 13 and 14, at which time the most severe effects occurred in the tail and the extremities. This Figure indicates that as the rectal temperature increases, so does the malformation frequency. Therefore, a significant rise in malformations and intrauterine

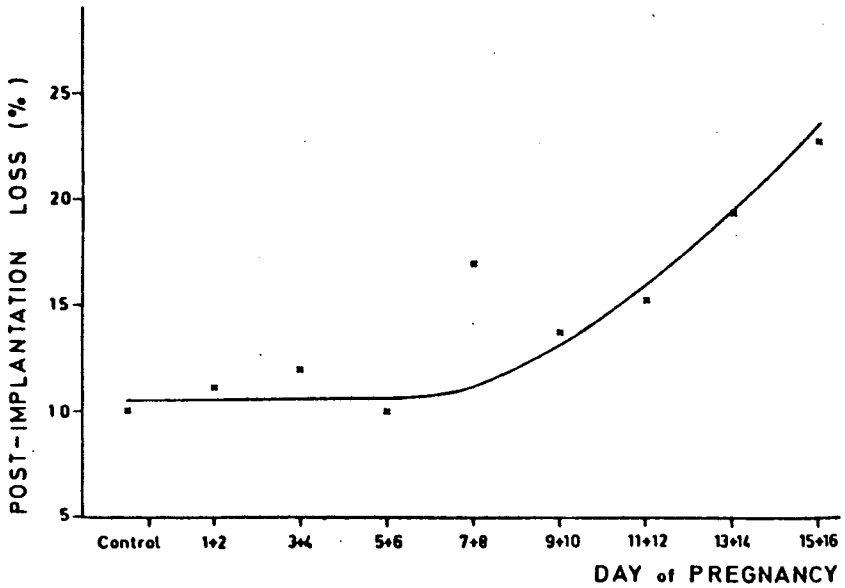

FIGURE 4. In later pregnancy stages (after completion of organogenesis), vhf irradiation is dangerous due to heat accumulation in the amniotic sac.

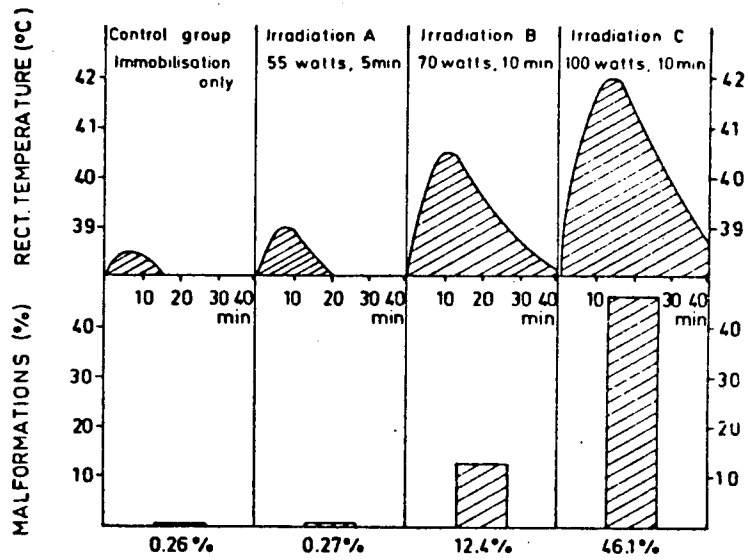

FIGURE 5. The frequency of malformations on Days 13 and 14 (tail and extremities) vs the rectal temperature curves of pregnant rats during vhf irradiation.

deaths is only observed when thermal effects occur. It should, however, be noted that contrary to tissues that are well perfused, in which the high-frequency energy delivered is quickly removed by the bloodstream, this does not occur in either the blastocyst prior to implantation or in the amniotic sac in later pregnancy. In such systems, considerable danger may be present due to heat accumulation. It is known that other little-perfused organs, especially liquid cavities (e.g., urinary bladder, tumor necrosis, and, a widely recognized example, the optic lens) are susceptible to selective overheating. The field intensities dangerous to embryonal cell development (e.g., vicinity of a transmitting aerial on a directional radio beam) have not been determined. It is not our opinion that athermic effects are significant for the embryo. However, we definitely believe that heat may accumulate, even in low field intensities.

DISCUSSION

Decrease in DNA Synthesis by vhf Radiation

Injuries to embryos have been thought to be caused by oxygen deficiency.[6,7] This belief was assumed in my previous papers[2–5] and in examinations by Menkhaus[8,9] of ^{35}S incorporation in fetuses. Moayer[10] suspects that there is injury to the placenta. In the final analysis, however, he believes that oxygen deficiency also plays a role in producing the injuries we have observed. Moayer's conclusions are derived from diffusion disturbances of the placenta.

We are presently treating tumors with vhf radiation. As tumor growth rate increases, so does the deoxyribonucleic acid (DNA) synthesis intensity. A method

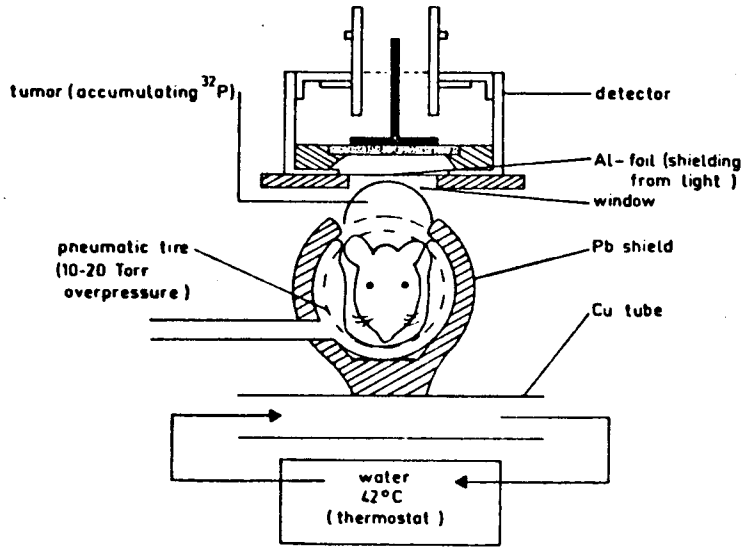

FIGURE 6. Extracorporeal measurement of ^{32}P incorporation rate as a quantitative indicator of the metabolic rate in proliferating mouse tumor. Based on Pfeiff *et al.*[13]

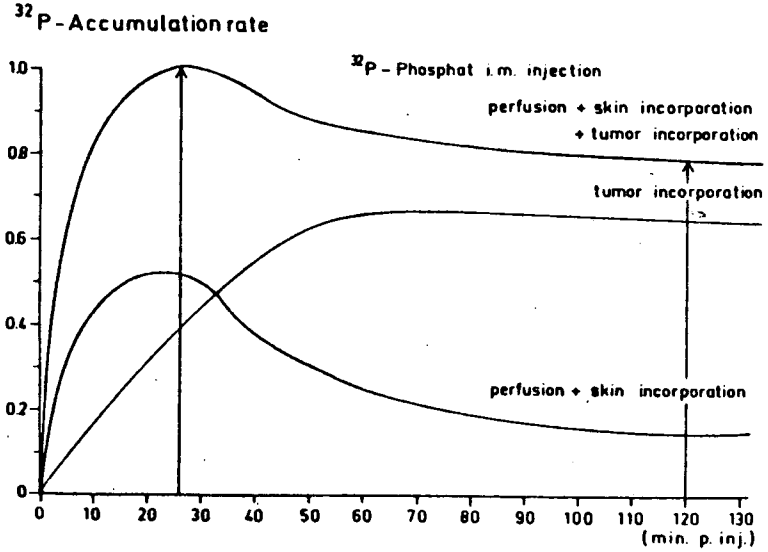

FIGURE 7. Tumor ^{32}P incorporation as determined by the 120-min value of ^{32}P uptake divided by the maximum uptake. Based on Pfeiff *et al.*[13]

used successfully, particularly in experimental oncology, is to determine the labeling index with [^{3}H]thymidine or [$^{131-125}$I]iododeoxyuridine.[11,12] The disadvantage of these methods is that examination material must be obtained by excision or puncture.

The advantage of the method developed by Pfeiff and associates[13] in our clinic during the last few years is that it is possible to follow tumor proliferation metabolism *in vivo*. Extracorporeal measurement of ^{32}P β-rays with semiconductor detectors yields a quantitative indicator of tumor proliferation kinetics (FIGURES 6 & 7).

Superficial experimental tumors treated with ionizing or nonionizing electromagnetic radiation present the following results: ^{32}P incorporation falls rapidly after the tumor is heated to 42°C with 461.04 MHz; this decrease is larger and more rapid than what we have observed after a 4000-r bilateral tangential irradiation (FIGURE 8).

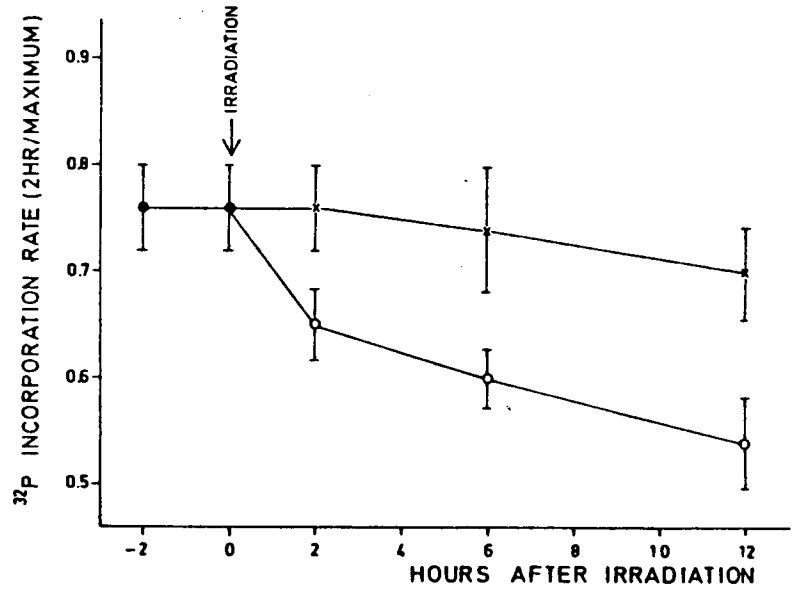

FIGURE 8. Tumor ^{32}P incorporation falls rapidly after vhf treatment. Contrary to this extreme decline, the decrease after a single 4000-r x-ray irradiation is slight in the first 12 hr. Data are as of February 1, 1974. ●, No irradiation; ×, 4000 r; ○, uhf.

Tumor DNA synthesis seems to be extremely thermosensitive. New results with [^{3}H]thymidine *in vitro* have already been obtained.[14-17]

If one compares the temperature curves yielded by the malformation experiments with the intratumoral temperature curves, one sees that they are quite similar (FIGURE 9). If DNA synthesis is interrupted at the point at which it is absolutely essential for replication, namely, during the phase of determination, a definite malformation results.

It is also known from autoradiographic experiments[18] that nucleic acid synthesis is extremely high, particularly during blastogenesis. In this phase, DNA present rises by a factor of 10 within 24 hr.[19] This fact explains the extreme sensitivity prior

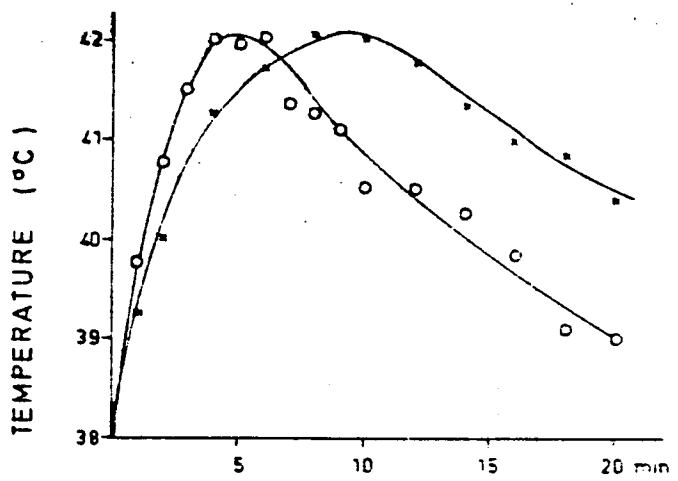

FIGURE 9. Comparison of the rectal temperature curve of pregnant rats to the temperature curve within neck tumors (Ehrlich) of mice. The heat produced by vhf irradiation causes malformations and also inhibits tumor growth. ×, Rectal temperature during irradiation of pregnant rats (27.12 MHz); ·, intratumoral temperature during irradiation (461.04 MHz).

to implantation of the blastocyst to an agent that terminates DNA synthesis, such as vhf radiation.

CONCLUSIONS

We have demonstrated that vhf radiation adversely affects embryonic development. We are certain, however, that eventually it will be possible to utilize high-frequency radiation to therapeutically treat malignant tumors.[20]

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DISCUSSION

DR. W. M. LEACH: It is very interesting that you have shown a large increase of anomalies through organogenesis. What is the duration of major organogenesis during prenatal development of the rat?

DR. DIETZEL: The organogenesis phase occurs during days 9-15 of gestation. After the 15th day, fetal genesis begins.

DR. S. M. MICHAELSON: I suggest that we hesitate to equate the effects observed with both types of radiation, because there are fundamental differences. With ionizing radiation, the effect is a direct consequence of energy absorption. With microwaves or heat, which apparently in this case are the same, the mechanism is entirely different. I am particularly concerned about effects on DNA synthesis.

DR. DIETZEL: I agree, but I think we could combine microwaves with x-radiation for their respective advantages in medical therapy.

DR. O. E. SCHOTTÉ (*Amherst College, Amherst, Mass.*): In the guinea pig, ovulation can be determined within minutes. The egg, which is free from the ovary, goes to the fallopian tubes, and takes four days to implant. During this time, the egg could be exposed to nonionizing radiation, and we would be able to determine whether it has any influence on implantation or whether implantation is possible or not under such circumstances.

DR. Z. R. GLASER (*Bureau of Medicine and Surgery, Bethesda, Md.*): Did you conduct any purely thermal studies?

DR. DIETZEL: I didn't compare the effect of microwave heating with other methods of heating.

DR. GLASER: The levels of microwave radiation that you used increased the temperature of your animals to 42°C. Others have found that this dose is extremely thermal and many of their animals have died during such irradiation.

DR. DIETZEL: We controlled the temperature continuously during irradiation. When the temperature reached 42°C, we had to remove the animals from the field; if we did not do this, they did not survive.

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