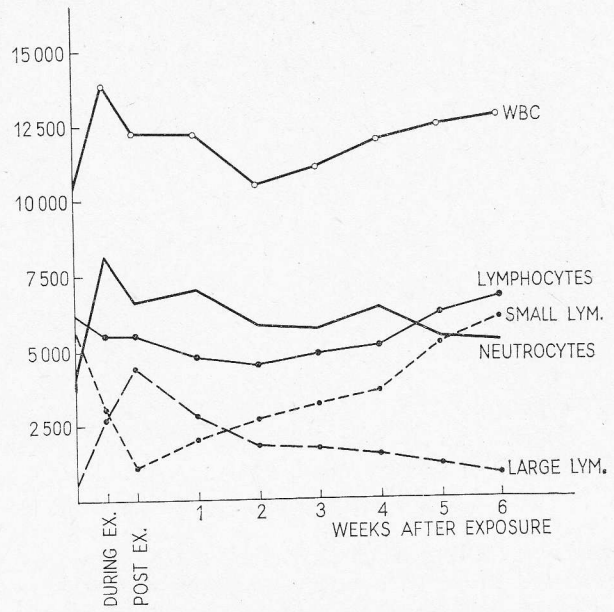

Fig. 4. Effects of microwaves on leukocytes.

more noticeable. Fibroblasts were found around the hemorrhagic and necrotic areas of the parenchyma.

In the 1st week after the end of the irradiation period, an increase in the exudate became more prominent and the fat cells decreased further. An overal reduction in cellularity was apparent and degeneration of the erythroid and myeloid cell was clearly seen. Degeneration of the blood vessels such as vacuolization also became more evident (Fig. 9).

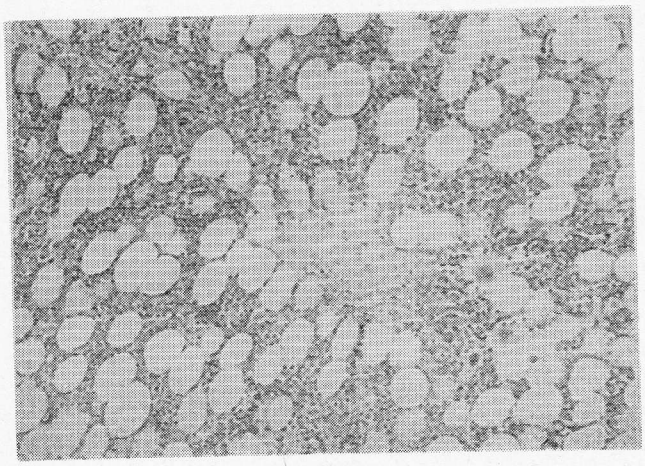

Fig. 5. Diffuse proliferative type. Diffuse proliferation of the hemocytoblasts is seen and plasma-like substances appear locally in the parenchyma. Irradiation 5 days, hematoxylin--eosin (×40).

WHO – Biologic effects & health hazards of microwave radiation 1974

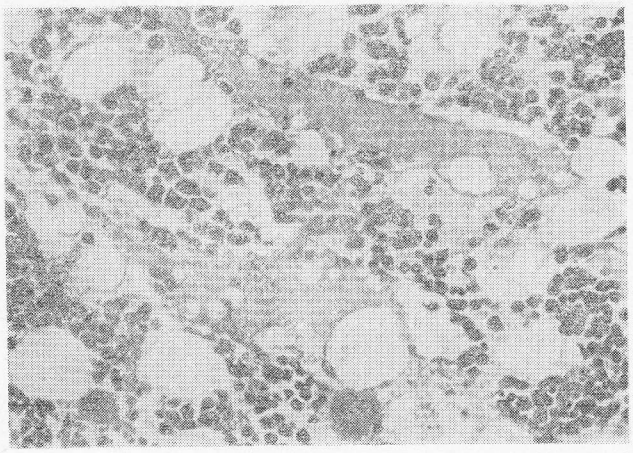

Fig. 6. The sinuses are filled with the plasma-like substances and dilated strongly. 3 days after irradiation, hematoxylin-eosin (× 120).

At the 2nd week, the fat cells disappeared due to an extreme increase in the exudate (Fig. 10). Degenerated parenchyma cells were scattered in the exudate like islands.

At the 3rd week, the exudative changes progressed further. Destruction and decrease of the parenchymal cells became more marked (Fig. 11). In some cases, partial fibrosis of the bone marrow was observed and completely fibrotic marrow was seen (Fig. 12).

At the 4th week, the exudate began to decrease and the fat cells began to appear again. However, the parenchyma cell decrease continued and severe degenerative changes were seen (Fig. 13). In some cases, aplastic bone marrow was seen, though little exudate remained between the fat cells.

In the 5th and 6th weeks, in some cases which showed remarkable exudation, distinct sinus wall thickening and plasmastasis in the sinus were seen. These sinus degenerations had a worm-like appearance (Fig. 14). However, in most cases, the exudate was absorbed and disappeared completely, while the fat cells increased progressively in place

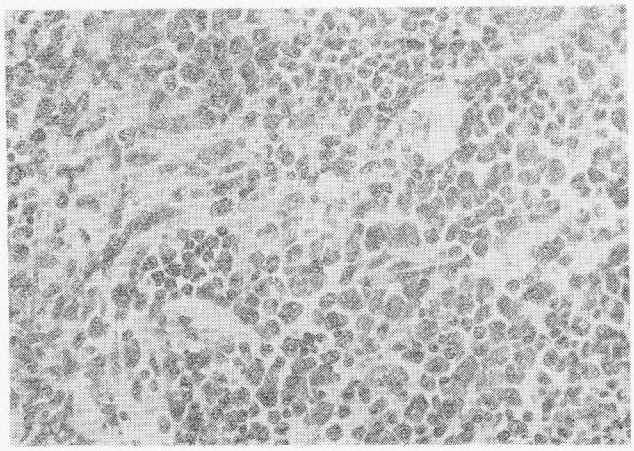

Fig. 7. Diffuse proliferation of the hemocytoblasts. Irradiation 7 days, hematoxylin-eosin (×120).

WHO - BIOLOGIC EFFECTS & HEALTH HAZARDS OF MICROWAVE RADIATION 1974

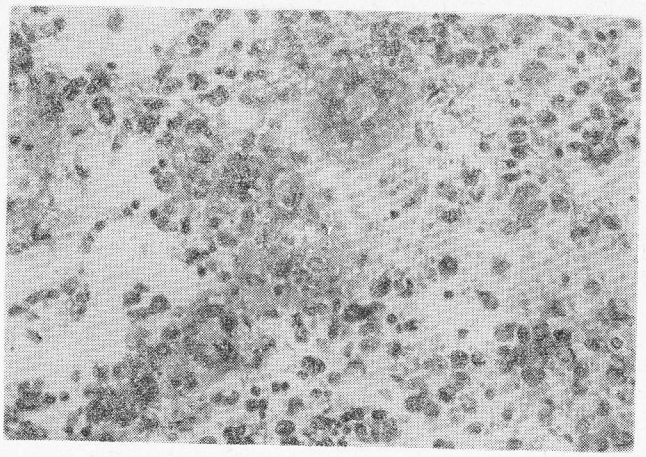

Fig. 8. Degeneration of the megakaryocytes. 3 days after irradiation, hematoxylin-eosin (×120).

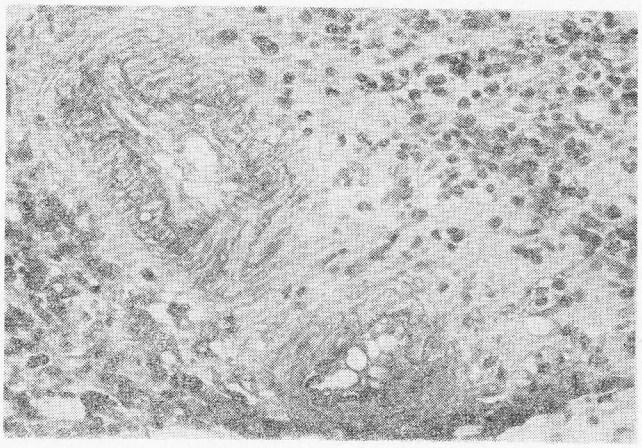

Fig. 9. Degeneration of the blood vessels and the plasma-like substances in the intravascular space. 1 week after irradiation, hematoxylin-eosin (× 120).

of the exudate. The parenchyma cells were destroyed and disappeared. Thus, completely aplastic bone marrow developed (Fig. 15). This aplastic bone marrow was almost the same as that found in human beings. Such completely aplastic bone marrow was found in 5 cases out of 7 in the 5th week and in 6 cases out of 9 in the 6th week.

However, in 2 cases out of 9 animals observed in the 6th week, slight regeneration of the hemocytoblasts was found (Fig. 16).

The fibrosis seen in the 3rd week after irradiation was not found in the bone marrow obtained in the 5th or 6th week.

c. Histochemical findings: In order to study the characteristics of the plasma-like amorphous substances in the parenchyma, blood vessels and megakaryocytes, the bone marrow preparations were stained by various methods, such as PAS, mucicarmine, Best's carmine, Van Gieson's silver nitrate, toluidine blue metachromasia at pH 3.5, Azan Mallory's and iron stainings (Tab. 3).

WHO – BIOLOGIC EFFECTS & HEALTH HAZARDS OF MICROWAVE RADIATION 1974

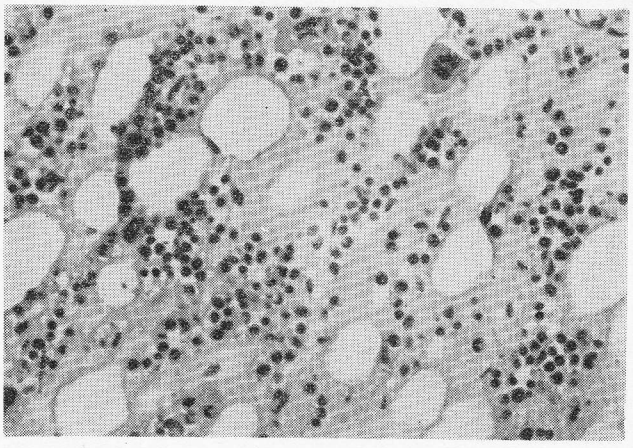

Fig. 10. So called "Gallert Mark" filled with the exudate. Parenchymal cells degenerate and decrease. 2 weeks after irradiation, hematoxylin-eosin (× 100).

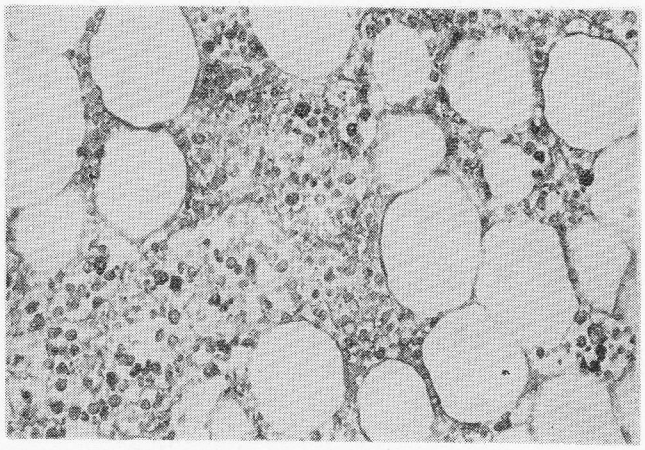

Fig. 11. The exudate is absorbed and the fat cells are increasing. Many degenerative cells and corpses of the cells are seen. 3 weeks after irradiation, hematoxylin-eosin (×120).

The plasma-like substance showed a positive reaction to PAS, with peaks within 1 week after the end of the irradiation period, and to TBM with a peak at the same time. Mucicarmine staining also became positive and showed a maximum in the 2nd week. From these findings, it was supposed that the plasma-like substance contained neutral and acid mucopolysaccharides. Therefore, the substance seemed to be an exudate which came from the blood serum due to an inflammation of the bone marrow.

The blood vessels also showed a positive reaction to PAS, Azan Mallory's and Van Gieson's stainings. A fibrinoid swelling was recognized in the blood vessels. From these observations, it seemed that the changes in the bone marrow after the irradiation were those of serous inflammation resembling allergic inflammation (2, 8).

The granulocytes and the megakaryocytes showed a positive reaction to PAS at the 1st week, but a very weak reaction from the 2nd week onwards and thereafter. These

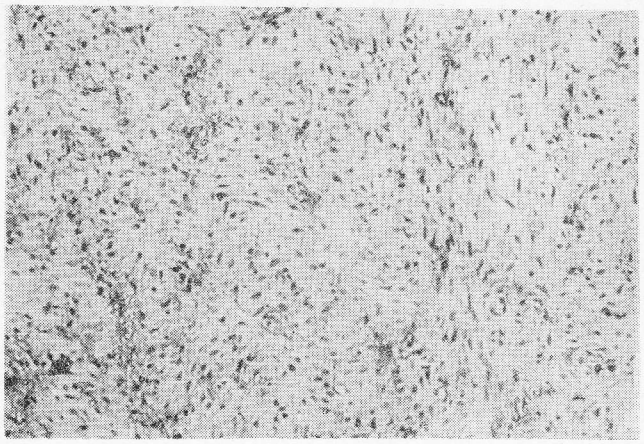

Fig. 12. Complete fibrosis. 3 weeks after irradiation, Azan Mallory's (×60).

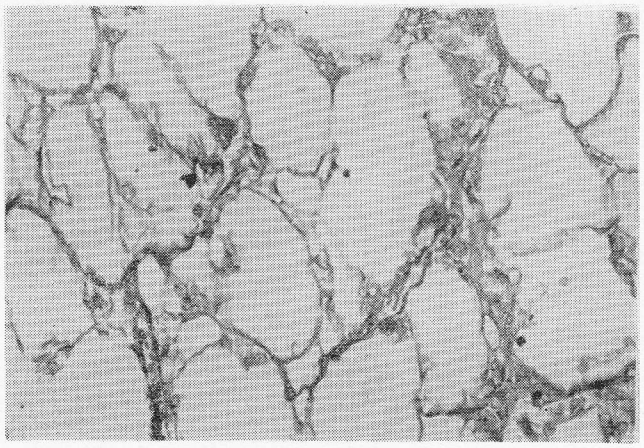

Fig. 13. Aplastic bone marrow. The exudate disappears and no parenchymal cells are seen. Notice the fat cells with irregular membranes torn off and elasticity lost. 4 weeks after irradiation, hematoxylin-eosin (×120).

findings seemed to suggest that the degeneration of these cells progressed from the 2nd week.

The cells containing the iron granules increased from the 1st to the 3rd week. This means that destruction of the erythrocytes occurred mostly in this period.

d. Alkaline phosphatase reaction of the sinus wall: From the results of the studies mentioned above, it appeared that microwave irradiation induced certain changes of the sinocapillary wall of the bone marrow and a serous inflammation, resulting in aplastic bone marrow.

In order to prove this sino-capillaropathy, Kurohane, one of our co-workers, studied the changes of the sinus wall by alkaline-phosphatase staining and the India ink infusion method.

Normal bone marrow showed a distinct positive reaction to AP staining. After the

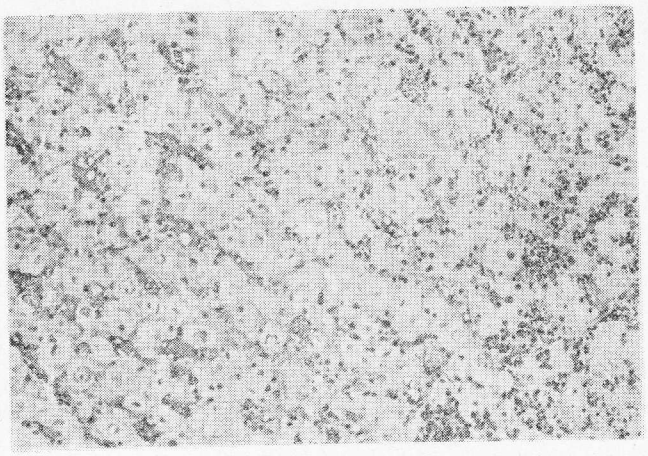

Fig. 14. Exudative changes are remarkable and plasma stasis in the sinuses is seen. 5 weeks after irradiation, hematoxylin-eosin (×120).

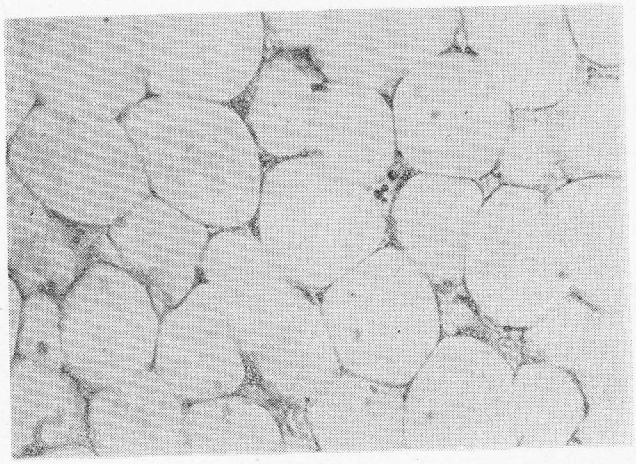

Fig. 15. A typically complete aplasia. The exudate and the hemocytoblasts disappear completely. The membranes of the fat cells become regular and show elasticity. 5 weeks after irradiation, hematoxylin-eosin (×120).

irradiation, the reaction decreased gradually. In the 2nd week, a weak reaction was still present, but thereafter the reaction was absent.

From the results of the studies, it was concluded that injuries of the sinus wall progressed after the irradiation, especially from the 2nd week.

5. Effects of cortisone on radiation-induced bone marrow changes: Ueyama (10), one of our co-workers, studied the effects of hydrocortisone on the changes of the bone marrow induced by microwave radiation.

In this experiment, 60 minutes of exposure to weak radiation was made once a day and continued for 14 days. The animals were divided into 3 groups, that is, a control group, a group treated daily with hydrocortisone from the beginning of irradiation and a group treated with hydrocortisone immediately after the irradiation period was terminated.

WHO – BIOLOGIC EFFECTS & HEALTH HAZARDS OF MICROWAVE RADIATION 1974

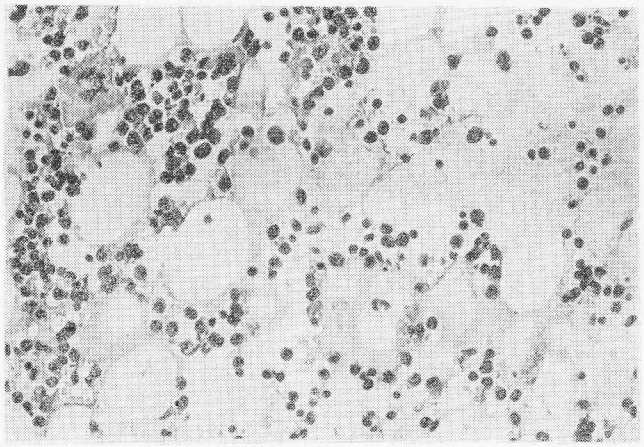

Fig. 16. The regenerative bone marrow. 6 weeks after irradiation, hematoxylin-eosin (×100).

In the control group, the exudative changes and destruction of the parenchymal cells occurred as mentioned above.

On the other hand, no such marked exudation occurred in the group treated with hydrocortisone from the beginning of the irradiation period and no changes were found in the parenchymal cells.

In the control group, aplastic bone marrow developed in the 3rd week after irradiation, but in the group treated with hydrocortisone, such an aplastic bone marrow did not develop. On the contrary, in some cases a proliferation of hemocytoblasts was found.

Even in the group treated with hydrocortisone immediately after the end of the irradiation period, exudative changes and degeneration of the parenchymal cells were very slight and aplastic bone marrow did not develop.

The results of the studies proved that bone marrow changes after irradiation consisted in serous inflammation which was suppressed by hydrocortisone administration.

DISCUSSION

In 1950, Umehara reported that microwave exposure produced a serous inflammation of many organs in guinea pigs, such as hepatitis, endo- or myocarditis, nephritis, pneumonitis, gastritis or even gastric ulcer. In that report he concluded that these changes might be caused by the harmful effects of microwaves on the endothelium of the capillary walls under rather low temperature. In the experiments reported here, it was proved that microwave radiation also caused sinus wall injuries in the bone marrow and induced a serous inflammation, resulting in aplastic bone marrow.

This indicates that microwave radiation has a clearly harmful effect on the bone marrow under certain conditions and aplastic anemia might be caused by an inflammation of the bone marrow rather than by stem cell failure (4).

REFERENCES

1. BARAŃSKI, S.: *Aerospace Med.*, 1971, **42**, 1196.
2. EPPINGER, H.: *Wien. Klin. Wchnschr.*, 1935, **48**, 665.
3. INOUE, N., FUJINO, K.: *Acta Haem. Jap.*, 1948, **11**, 140.
4. JANDLE, H.: *Medicina*, 1964, **43**, 615.
5. KICOVSKAJA, I. A.: *Aerospace Med.*, 1965, **36**, 182.
6. MICHAELSON, S. M., THOMSON, A. E., HOWLAND, J. W.: *Am. J. Physiol.*, 1961, **201**, 351.
7. MICHAELSON, S. M.: et al.: *Aerospace Med.*, 1964, **35**, 824.
8. RÖSSLE, R.: *Virch. Arch.*, 1933, **291**, 1.
9. SASADA, S.: *Denpa Hippon*, 1947, **42**, 25.
10. UEYAMA, R., et al.: *Acta Haem. Jap.*, 1972, **35**, 262.
11. UMEHARA, S.: *Nippon Acta Radiologica*, 1950, **10**, 1.
12. YAGI, K.: *Nippon Acta Radiologica*, 1970, **30**, 184.

WHO – Biologic effects & health hazards of microwave radiation 1974

EFFECTS OF MICROWAVES ON THE CELL METABOLISM OF THE RETICULO-HISTOCYTIC SYSTEM

L. Miro, R. Loubière and A. Pfister

Faculty of Medicine in Montpellier-Nimes, Laboratory of Biological Physics, Nimes Section, Nimes, France

From clinical and biological investigations of people exposed to microwaves, we found that an exposure for a long time at a low mean power density (from 0.1 to 0.2 mW/cm^2) produced subjective and objective disorders in a number of subjects.

In order to find out the source of these disorders, a first series of experiments was carried out with material to be described later. During 450 hours, mice, rats and rabbits were exposed to the action of continuous microwaves of 7 mW/cm^2 mean power density. During the experiment no modification in either the behaviour or appearance of the animals could be noted. At the end of this experiment all the animals were decapitated and anatomo-pathologic studies of all their organs were made. No macroscopic or microscopic alteration of these organs could be noted; we found only a hyperplasia of the spleen and liver of these animals which seemed to indicate a possible stimulation of their reticulo-histocytic system.

With the aim of investigating this effect in more detail we carried out a systematic study on the liver, spleen and thymus cell metabolism of mice exposed to microwaves.

MATERIAL AND METHODS

Exposure to microwaves was carried out using a pulse-modulated emitter with the following characteristics:

— wavelength: 10 centimeters
— frequency: 3.105 MHz ± 15 MHz
— pulse width: 1 ms
— duty cycle: 50 Hz
— emitting peak power: 400 W
— emitting mean power: 20 W.

The microwaves were emitted from a horizontal horn and the exposed animals were situated in the far field where the mean power density was about 2 mW/cm^2.

All the experimental animals (Fig. 1) were situated in an anechoic chamber 3 meters in diameter, made of absorbant Eccosorb 330 F.R. sheets, the power measurement being carried out with a Sperry field meter.

The experiments were carried out on 40 male mice of "Swiss" pure strain, weighing from 26 to 30 grams. Each mouse was identified and matched with another of the same weight to haft a gram. Thus, two identical groups were constituted, one being used as a control group and the other exposed to microwave radiation.

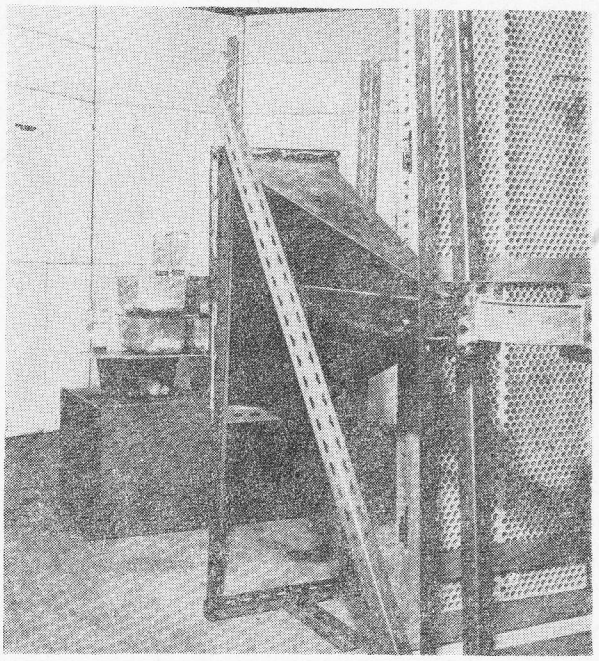

Fig. 1. For explanation, see text.

The experimental group was continuously exposed during 145 hours to microwave radiation, the control group being kept under exactly the same conditions of life in another room throughout all the experimental period;

The animals were not restrained and received water and food at will.

The weight of each animal was recorded daily as well as its behaviour; we also controlled the central temperature of the animals, maintaining it stable so that it did not vary more than half a degree centigrade.

After the exposure we injected all the animals intraperitoneally with a methionine ^{35}S solution in a dose of 2.5 μCi per gram of body weight, and each of the groups was returned to its appropriate surrounding, one normal, the second microwave irradiated.

15 hours after the injection, all mice were decapitated and an autopsy was made immediately. The spleen, liver and thymus were set apart and fixed in "Bouin liquid", then treated according to the classical anatomo-pathologic techniques. However, an inclusion in paraffin was performed, the homologous organs of each matched animal (the control and the experimental one) being included in the same block. Thus on the same microscopic preparation one could find side by side a view of an experimental and a control organ having exactly the same thickness and processed in the same manner.

Each preparation was then covered with nuclear emulsion Ilford (G5), dried and preserved in a cold and dark place for 15 days, and then processed.

The optical density obtained after the reduction of the silver salt by the 167 keV beta radiation of ^{35}S methionine was measured with a "Lison histophotometer" coupled to a Reichert-Zetopan microscope. Comparative measurements were made on the homologous preparations by the examination of corresponding sites; each value is the statistical result of 30 measurements.

WHO - BIOLOGIC EFFECTS & HEALTH HAZARDS OF MICROWAVE RADIATION 1974

By another method hematein-eosin-safran staining was performed simultaneously on a preparation coming from the same paraffin blocks as those from which the preparations for histoautoradiography were made. So it was possible to compare the classical anatomo-pathologic aspect of the control and exposed organs and to locate histologically the incorporated ^{35}S methionine.

RESULTS

During irradiation no difference between the behavior of control animals and exposed animals was noted, and weight curves did not show any modification in the initial weight.

At the time of autopsy, three matched animals were rejected from the experiment because they developed certain organic lesions without clinical manifestations and these might have affected the incorporation of amino acids.

As the end result 17 matched animals were studied.

The classical anatomo-pathologic examinations permitted the following conclusions:

Spleen. There was a greater extension of germinative centers in exposed animals than in controls; the cellular density increased and lymphoblastic cells were more numerous (Fig. 2).

In lymphoid areas the lymphocytes were abnormally distributed in sheets; foci of eosinophil cells and large numbers of reticular cells were found.

In the red pulp many of the same cells could be seen completing the picture of spleen hyperplasia.

Disorganization of the hepatocytic structure, and enlargement of the hematopoietic islet were also observed.

Liver. An increase of the reticular and histocytic cells of mesenchymatic origin in the Disse spaces was noticed (Fig. 3).

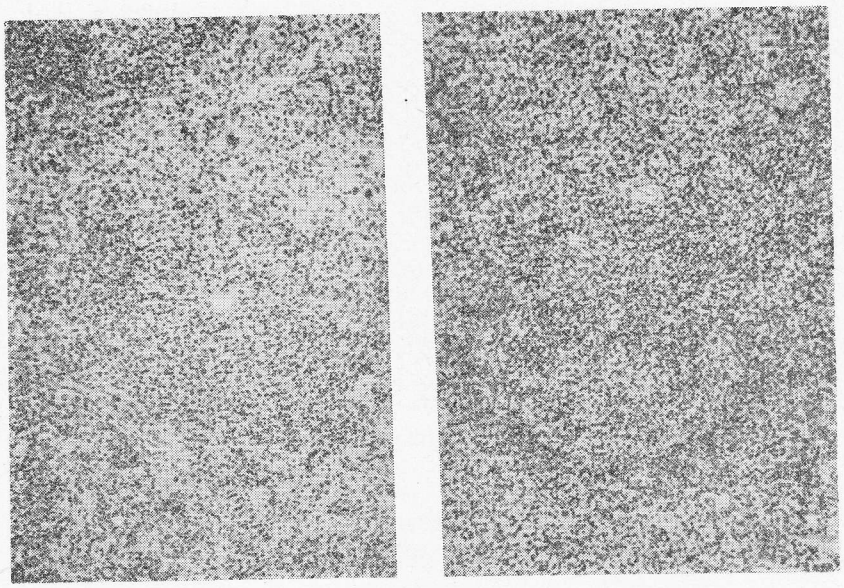

Fig. 2. For explanation, see text.

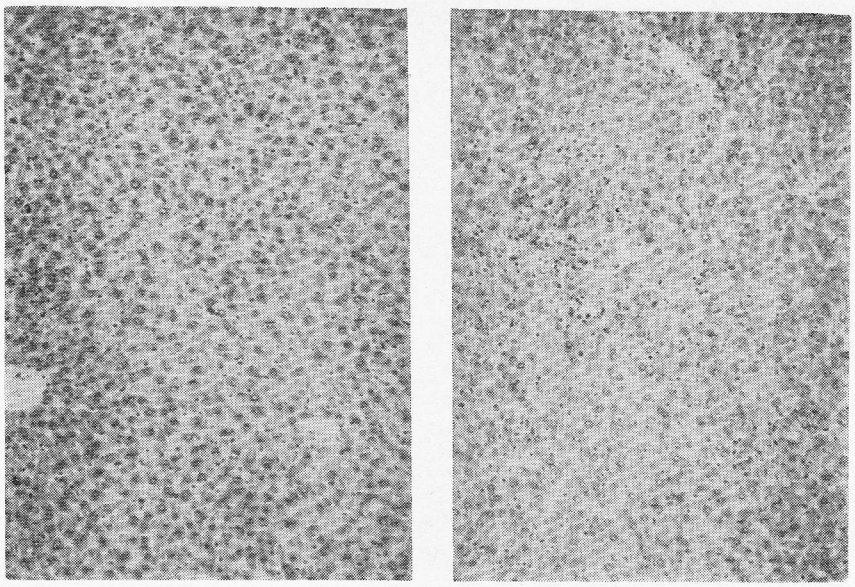

Fig. 3. For explanation, see text.

Thymus. The cellular density of cortical areas was marked in the subcapsular zones by a great number of lymphoblasts and abundancy of reticular cells.

This reticulohistiocytic hyperplasia was confirmed by the dynamic examination of these organs, i.e. the study of ^{35}S methionine incorporation. The optic densities of hepatic preparations and the homologuos splenic and thymic zones were measured and compared between control and exposed animals.

For each group a good gaussian distribution of results was obtained which permitted a statistical analysis to be made using Student's "t" test.

In Tables 1, 2, 3, 4, for each pair of animals the average measurements of optic density, the values of "t" and the degree of significance are given.

For the germinative centers and lymphoid zone of the spleen, liver and thymus, both in the cortical zone and in the medulla, we observed in all the exposed animals a very clear increase in the rate of incorporation of the tagged aminoacid as compared to the control animals (Fig. 4). This difference is very highly significant in all cases ($p < 0.001$). The magnitude of the increase obtained is variable and seems to be connected with an individual factor.

Only in the red pulp of the spleen could a wider range of results be seen: if in most cases (88% of animals) the ^{35}S methionine incorporation is higher in exposed mice, the statistical studies show that the average difference is very significant ($p < 0.001$) only in 12 animals and that the degree of significance varies between 0.001 and 0.005 in 3 animals; in at least 2 cases the average difference is not significant.

At the end of the first part of the study we noticed an increase of ^{35}S methionine incorporation in the spleen, the liver and the thymus of mice exposed to microwave radiation.

These results pose three kinds of problem:

— the significance of the optical density measured histophotometrically,
— the limitations of the method used,
— the overall possible interpretation of the results.

WHO - Biologic effects & health hazards of microwave radiation 1974

Table 1

Incorporation of ^{35}S methionine into the germinative centers of the spleen (measurements of optical densities)

Pair of animals No.	Averages in animals		t	Degrees of significance
	exposed	controls		
1	19.25	13.88	12.17	$0.001 > p$
2	19.00	14.63	11.64	$0.001 > p$
3	13.50	11.75	3.78	$0.001 > p$
4	19.76	8.63	11.00	$0.001 > p$
5	19.20	9.56	18.00	$0.001 > p$
6	32.00	21.00	10.67	$0.001 > p$
7	26.40	18.00	16.50	$0.001 > p$
8	21.00	9.33	16.31	$0.001 > p$
9	30.93	18.73	21.07	$0.001 > p$
10	29.00	18.83	20.62	$0.001 > p$
11	27.63	22.93	8.62	$0.001 > p$
12	21.40	17.56	10.69	$0.001 > p$
13	25.36	18.76	6.16	$0.001 > p$
14	23.43	21.06	4.83	$0.001 > p$
15	28.90	26.36	4.23	$0.001 > p$
16	33.86	26.36	10.70	$0.001 > p$
17	28.20	23.33	8.10	$0.001 > p$

Table 2

Incorporation of ^{35}S methionine into the lymphoid zone of the spleen (measurements of optical densities)

Pair of animals No.	Averages in animals		t	Degree of significance
	exposed	controls		
1	16.50	13.33	4.50	$0.001 > p$
2	8.85	6.90	5.81	$0.001 > p$
3	8.80	6.70	4.66	$0.001 > p$
4	11.33	3.83	7.07	$0.001 > p$
5	10.06	6.03	9.60	$0.001 > p$
6	19.33	15.00	7.21	$0.001 > p$
7	17.00	11.00	12.14	$0.001 > p$
8	11.40	4.56	13.00	$0.001 > p$
9	18.50	12.33	6.02	$0.001 > p$
10	18.13	12.00	14.46	$0.001 > p$
11	13.03	10.83	8.50	$0.001 > p$
12	11.83	9.17	12.60	$0.001 > p$
13	15.76	11.96	11.27	$0.001 > p$
14	14.66	13.73	9.52	$0.001 > p$
15	20.63	16.26	9.23	$0.001 > p$
16	23.46	16.26	13.28	$0.001 > p$
17	16.03	12.70	9.68	$0.001 > p$

Table 3

Incorporation of ^{35}S methionine into the spleen red pulp (measurements of optical densities)

Pair of animals No.	Averages in animals		t	Degree of significance
	exposed	controls		
1	32.10	23.56	7.77	$0.001 > p$
2	15.89	14.21	2.10	$0.05 p > 0.02$
3	15.00	15.53	0.70	insignificant
4	22.53	9.00	24.00	$0.001 > p$
5	16.93	12.13	6.00	$0.001 > p$
6	33.00	34.00	0.67	insignificant
7	32.80	28.00	2.46	$0.02 > p > 0.01$
8	22.90	9.50	15.20	$0.001 > p$
9	32.86	27.26	4.44	$0.001 > p$
10	28.90	20.53	12.96	$0.001 > p$
11	22.23	20.26	5.18	$0.001 > p$
12	18.26	16.16	3.48	$0.001 > p$
13	30.36	27.46	3.76	$0.001 > p$
14	25.30	23.73	3.07	$0.01 > p\ 0.01$
15	31.43	26.70	7.70	$0.001 > p$
16	35.10	26.70	12.27	$0.001 > p$
17	26.83	23.26	6.81	$0.001 > p$

Table 4

Incorporation of ^{35}S methionine into the liver (measurements of optical densities)

Pair of animals No.	Averages in animals		t	Degree of significance
	exposed	controls		
1	16.46	13.06	6.12	$0.001 > p$
2	13.73	9.66	8.02	$0.001 > p$
3	13.60	11.36	4.75	$0.001 > p$
4	13.66	6.36	12.89	$0.001 > p$
5	13.66	9.10	6.66	$0.001 > p$
6	18.53	8.60	15.11	$0.001 > p$
7	14.46	11.46	5.07	$0.001 > p$
8	14.50	7.03	15.36	$0.001 > p$
9	15.53	9.63	12.36	$0.001 > p$
10	15.03	9.83	13.00	$0.001 > p$
11	17.33	12.16	14.60	$0.001 > p$
12	20.93	18.48	6.21	$0.001 > p$
13	31.76	28.93	4.12	$0.001 > p$
14	17.20	11.60	7.88	$0.001 > p$
15	26.30	21.73	4.52	$0.001 > p$
16	24.93	12.93	30.60	$0.001 > p$
17	24.72	21.30	7.96	$0.001 > p$

WHO - BIOLOGIC EFFECTS & HEALTH HAZARDS OF MICROWAVE RADIATION 1974

Table 5

Incorporation of ^{35}S methionine into the thymus (measurements of optical densities)

Cortical zone

Pair of animals No.	Averages in animals		t	Degree of significance
	exposed	controls		
2	24.70	21.80	5.00	$0.001 > p$
4	20.56	8.65	26.71	$0.001 > p$
5	18.46	10.40	23.79	$0.001 > p$
6	17.23	10.13	14.13	$0.001 > p$
7	15.83	11.53	10.14	$0.001 > p$
8	15.16	10.40	11.24	$0.001 > p$

Medullar zone

Pair of animals No.	Averages in animals		t	Degree of significance
	exposed	controls		
2	14.43	10.60	11.44	$0.001 > p$
4	11.53	5.50	17.48	$0.001 > p$
5	12.40	6.13	13.36	$0.001 > p$
6	12.26	6.66	15.77	$0.001 > p$
7	9.93	6.83	9.81	$0.001 > p$
8	10.60	6.93	10.15	$0.001 > p$

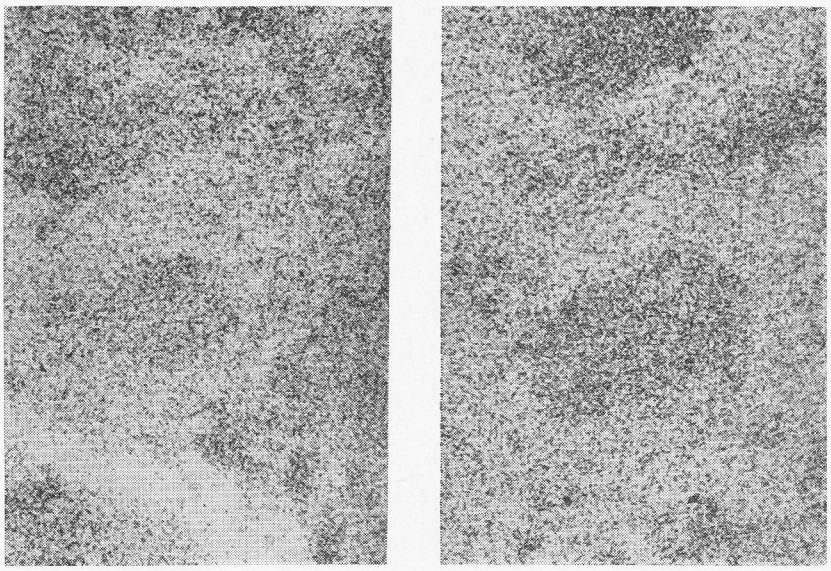

Fig. 4. For explanation, see text.

WHO – BIOLOGIC EFFECTS & HEALTH HAZARDS OF MICROWAVE RADIATION 1974

The technical protocol of our experiment allows us to give an answer to the first question. It may be demonstrated that, by using the histologic technique described, 91 to 97% of the radioactivity is due to the radiation from sulphur-35 of the methionine incorporated into a protein. The increased radioactivity observed in the mice exposed to microwave radiation is in conformity with an increase in protein synthesis by hepatic, thymic and splenic cells.

This is not a simple slowing down of the protein metabolism rate, but a real increase in the synthesis itself. In fact, in view of the relatively long time (15 h) which elapsed between the amimoacid administration and the sacrificing of the animals, we may consider that a steady metabolic state was established.

Finally, having included in the same paraffin block the organs of the matched mice, it was not possible to determine the differences between the optic density of the two preparations. Indeed these strictly underwent the same autoradiographic technologic processing: their thickness was the same, they were covered with the same emulsion film and the exposure time was the same.

The discordant results obtained in the red pulp could be explained by its anatomic nature: indeed, it is composed of many blood vessels. The radioactivity of this area depends not only on the Billroth strand, but also on the blood contained in sinusoid capillaries and venous sinuses. This fact explains why the blood radioactivity is modified according to the spleen contraction state at the moment of decapitation.

The limits of the method used result essentially from the choice of the labelled radioactive marker: the ^{35}S methionine indicates in fact only the protein cellular synthesis. All the cells contain sulphur proteins. This method does not allow differentiation of the histologic type of cells whose protein metabolism is increased. The resolution of histoautoradiographic methods does not allow by itself such a distinction. Therefore it is necessary to compare the hematein-eosine-safran stained preparation and the homologous autoradiographic preparation, each one coming from the same paraffin block. In this way we may demonstrate that the zone where the incorporation of ^{35}S methio-

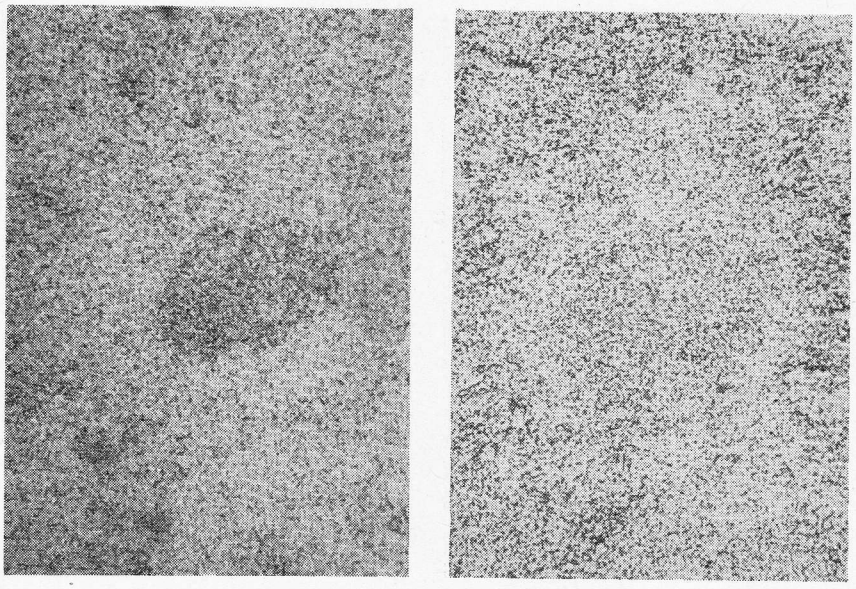

Fig. 5. For explanation, see text.

WHO – BIOLOGIC EFFECTS & HEALTH HAZARDS OF MICROWAVE RADIATION 1974

nine is increased corresponds to that zone where an increase in reticular and histiocytic cells is noted. Thus, in the spleen the more active zones in histoautoradiographic preparations are localized in the middle of germinative centers where a greater number of cells, especially lymphoblastic cells, is present (Fig. 5).

In the same way for the thymus a correlation can be made between the presence in the subcapsular zone of a large number of lymphoblasts with abundance of reticular and histiocytic cells and the existence in this zone on histoautoradiographic preparations of a darker strip than the remainder of the preparation.

In this way we were able to demonstrate histoautoradiographically an increase in protein synthesis in the zone where a histologically histocytic hyperplasia is noted.

CONCLUSION

In the actual state of our research we may conclude that the continuous exposure of mice for 160 hours to microwave radiation induces a great increase in protein synthesis in the liver, thymus and spleen. This was observed in zones where reticular hyperplasia was demonstrated by classical anatomo-pathologic methods.

In order to confirm or invalidate this effect of microwave radiation on the reticulo-histocytic system, it will be necessary to use a radioactive labelled product having a specific affinity for this system, such as colloidal gold-98 or a denatured iodine-125 albumin complex.

ARE MICROWAVES TERATOGENIC?

R. Rugh, E. I. Ginns, H. S. Ho, and W. M. Leach

Division of Biological Effects, Bureau of Radiological Health, Food and Drug Administration, United States Public Health Service, Rockville, Maryland, U.S.A.

INTRODUCTION

Accurate and reproducible dosimetry is proving to be a most important prerequisite for significant biological experimentation with microwave radiation. Since a dose-biological effect relationship should be established with experiments where the absorbed dose can be described, we have developed in our laboratory a waveguide irradiation facility that permits the precise determination of the total energy absorbed by a freely moving small living animal. The energy absorbed by an animal is dependent upon the geometry, size, orientation, and complex dielectric constant, and very possibly also upon such biological variables as hydration state, age, and endocrine status of each animal. Thus, two animals exposed to the same forward power of microwaves would most probably not absorb the same amount of energy in the same amount of time.

The extent of heat stress in animal systems during microwave irradiation has been evaluated by several workers. Haines and Hatch (3) as well as Belding and Hatch (1) proposed that heat loss and heat gain due to convection, the subject's metabolic rate, and the heat gain due to radiation, all contribute to the load experienced by a subject in the microwave field. In an effort to describe quantitatively the physiologically stressed state of a standard man in a microwave field, Mumford (5) derived values for the Heat Stress Index as a function of the Temperature Humidity Index (THI) and power density. Using rabbits, dogs, mice, and rats, Deichmann (2) further investigated the relationships between an animal's survival in a microwave field and the temperature, humidity, and air flow in the environment. These studies have shown that environmental conditions can influence the extent of stress upon any animal in a microwave field. Therefore, meaningful microwave biological effects data must be obtained from exposure situations where the environmental variables have been recognized, defined, and included in the dosimetric data. Our facility takes environmental variables into account to determine the integral absorbed dose of each animal, since it is the absorbed dose rate and dose that is of biological significance.

MATERIALS AND METHODS

Irradiation apparatus:

A detailed description of both the dosimetric and environmental parameters of our irradiation facility has been presented previously by Ho, Ginns and Christman (4). A chamber surrounding the waveguide permits the control of temperature, relative humidity, and air flow past the animal during exposure to microwave radiation. This chamber is shown schematically in Figure 1. The environment within the chamber is obtained by mixing two air streams — one that has been cooled and dehumidified, and

WHO – BIOLOGIC EFFECTS & HEALTH HAZARDS OF MICROWAVE RADIATION
1974

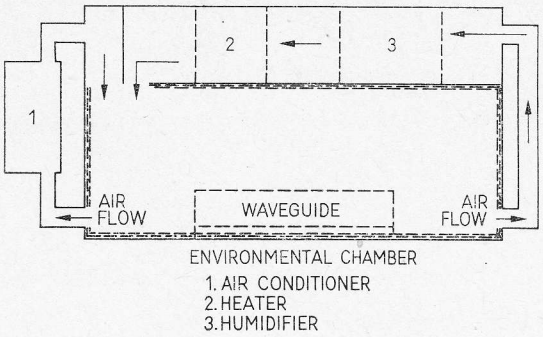

Fig. 1. Schematic drawing of the waveguide irradiation apparatus with controlled temperature and relative humidity environment. Conditioned air is drawn through the waveguide by means of a vacuum system.

one that has been heated and humidified. The air temperature is measured with a protected thermocouple probe located at the center of the chamber just above the waveguide. The range of relative humidity (measured with a membrane hygrometer at the air inlet of the waveguide) that can be obtained within the chamber is 25 to 70 percent ± 1.5 percent over the temperature range of 15 to 40°C, ± 0.5°C. Using the following equation by Mumford (5):

$$\mathrm{THI} = 1.44\ \mathrm{T} + 0.1\ \mathrm{RH} + 30.6$$

(where T = temperature in °C; RH = relative humidity) the environmental chamber provides a range of THI from 55 to 95. The temperature- and moisture-conditioned air within the chamber is drawn through the waveguide by a low-pressure (vacuum) system attached to one end of the waveguide.

The microwave generator provides up to 100 watts of continuous wave power at 2450 MHz. Within the waveguide the test animal is free to move within a holder (4.30 × 4.50 × 2.15 inches) made of low-loss materials (polyethyelene and styrofoam), which accounts for a 40 percent loss of forward power by reflection and absorption. Holes in the ends of the holder permit air flow past the animal.

The total amount of microwave energy absorbed by the animal is determined without perturbing the microwave field interacting with the irradiated animals. The forward, reflected, and transmitted powers are measured by power meters. The voltage output from each of the three power meters is fed into an integrator. Each of the power readings is integrated with respect to time and the integral dose can subsequently be calculated. The terms which describe dosimetric quantities are as follows:

Exposure rate: Incident power density (mW/cm²) of an electromagnetic wave.

Integral dose rate: Time rate of absorption of electromagnetic energy (watts) by the complete biological target.

Integral dose: Total amount of electromagnetic energy (joules or calories) absorbed by the complete biological target.

Average dose: Integral dose per unit weight of the animal (joules/g or cal/g).

Distributed dose rate: Time rate of absorption of microwave energy per unit mass (W/kg or W/g). It is usually non-uniform in biological bodies even though the incident power density (exposure rate) may be uniform.

Since the animal is free to move within the holder during exposure in the waveguide, the reflected and transmitted powers are constantly varying. A typical pattern of reflected power variation during an exposure is illustrated in Figure 2. The ordinate

WHO – Biologic effects & health hazards of microwave radiation 1974

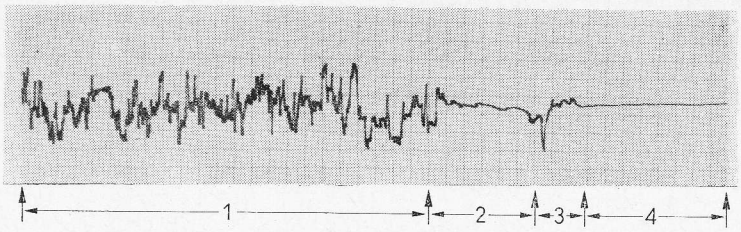

Fig. 2. Tracing of power reflected from an unanesthetized animal. These traicings provide a monitor of animal movement. Four phases of animal activity can be defined, as indicated on the chart.

corresponds to the voltage output from the power meter. The power variation can also be used as an "activity indicator" of the animal during exposure. It provides a means of investigating the sensitivity of an animal to both environmental and dosimetric (e.g., dose rate) parameters for which lethality is the biological endpoint. Death of the irradiated animal can be determined and the average integral or absorbed dose to death can be calculated.

Biological materials: Mature CF_1 white mice, 25—30 g, were used in these experiments. Females were timed-mated for the fetal studies, so that 8.5-day gestation stages were provided for microwave irradiation. Gestation day 8.5 is known to be particularly sensitive to ionizing radiation with respect to anomaly production. Some pregnancies were allowed to come to term, but most were terminated at 18 days to examine whole litters before the mother had an opportunity to destroy any of her malformed young, which is common practice for the mouse. Thus, in those graphs depicting fetal studies each point represents an entire litter, categorized as to percentage of anomalies, resorptions, etc. The following data represent the beginning of studies designed to compare the effect of ionizing and microwave radiations on the fetus. Such a comparison is of interest since ionizing radiations involve little or no rise in body temperature, whereas microwave radiation can involve substantial body heating.

EXPERIMENTAL RESULTS AND DISCUSSION

The irradiation of pregnant mice was performed with a forward power of 7.37 watts, constant temperature of 25°C and relative humidity of 50 percent, with variable exposure durations up to a maximum of 5 minutes. The THI calculated for these environmental conditions was 71.6. Prior to the initiation of irradiation, each mouse was acclimatized to this environment for 30 minutes. The average dose varied between approximately 3 and 8 cal/g. As can be seen in Figure 3, the average dose for lethality of adult male mice at a THI of about 70 is 12 cal/g (experimental conditions: forward power, 6.94 watts; variable temperature and relative humidity; duration of irradiation until death, as shown in Figure 3). Differences in response to microwave radiation are expected because of such factors as activity, sex, state of hydration, age and health status; and both integral dose rate, and dose distribution in the animal. These differences

WHO – BIOLOGIC EFFECTS & HEALTH HAZARDS OF MICROWAVE RADIATION 1974

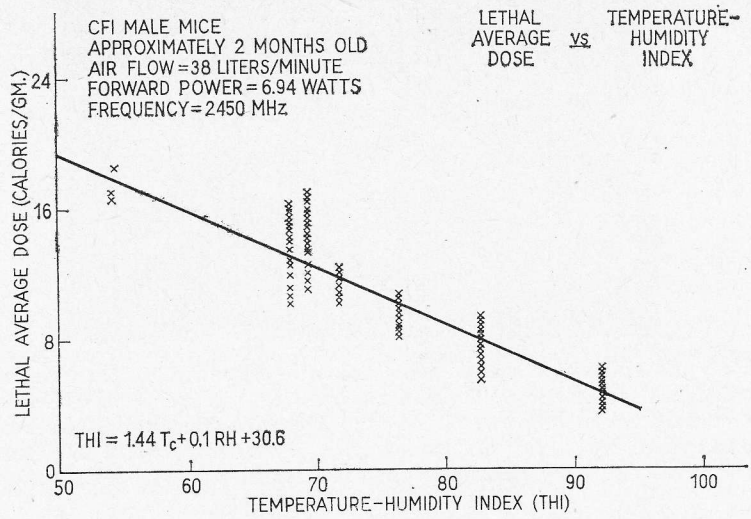

Fig. 3. The relationship between average dose to lethality of male mice and the temperature-humidity index (THI). Each point represents a single exposed animal. The THI formula and other experimental conditions are shown in the Figure.

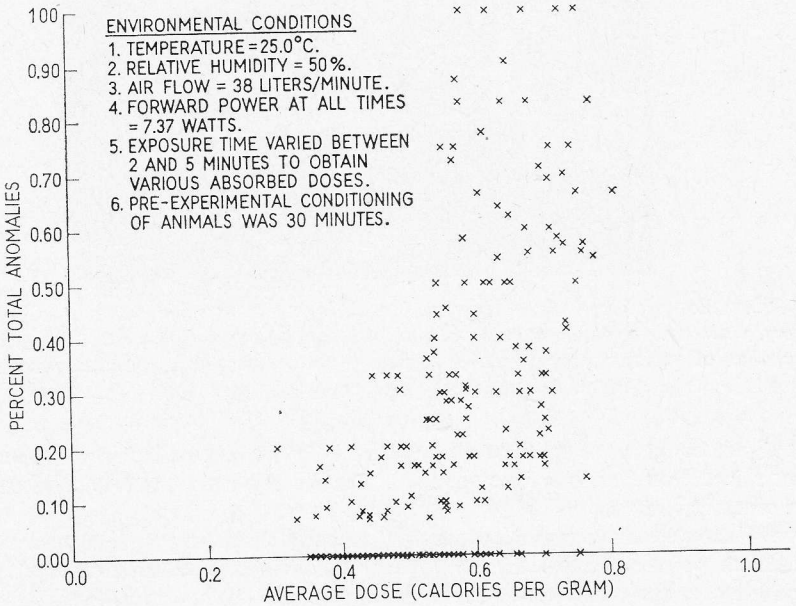

Fig. 4. Total observed fetal anomalies from microwave exposure. Each point represents the fraction of anomalous offspring in each irradiated litter, and is expressed as a percent of the litter. Environmental exposure conditions are as stated.

WHO – Biologic effects & health hazards of microwave radiation 1974

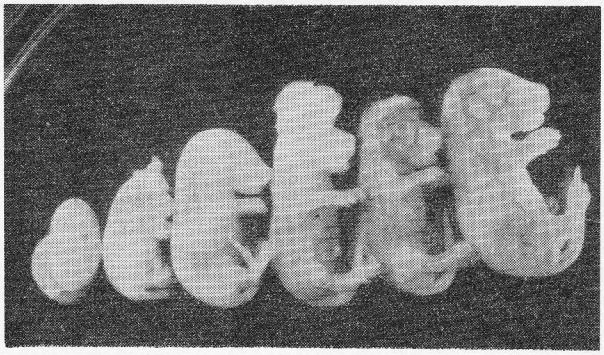

Fig. 5. Variety of anomalies obtained from various litters which had been exposed to microwave radiation. From left to right: Dead fetus still attached to placenta. Dead fetus with almost anencephaly. Apparently normal but stunted fetus. Normal sized fetus but with pronounced exencephaly. Ice-pack type exencephaly, with circulatory stasis. Apparently normal fetus with circulatory stasis.

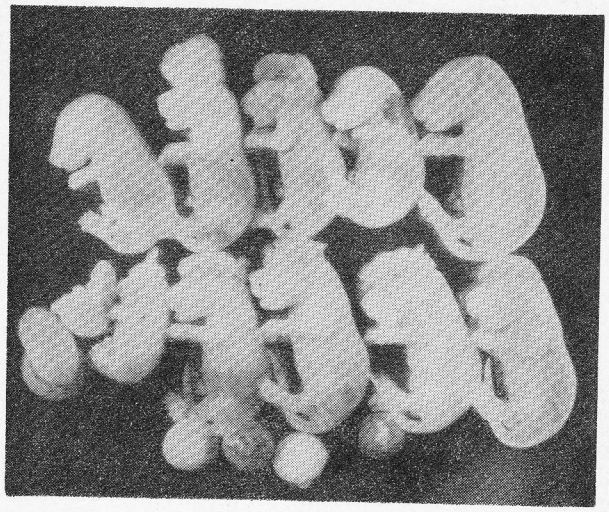

Fig. 6. Anomalies produced by microwave irradiation of CF_1 mice on their eighth day of pregnancy, showing: Resorptions (three examples). Dead fetuses (three examples), lower left. Exencephalies of various degrees (six). Stunting — (two examples); apparently normal topographically. Normal (one example) upper right.

prevent direct comparison of the data in Figure 3 with the radiation absorbed by the pregnant mice. However, we concluded that the doses to pregnant mice were moderately to severely stressful.

Figure 4 shows total observed anomalies among fetuses exposed to microwave radiation. Each point refers to the proportion of anomalous fetuses observed in each dissected litter, and is expressed as a percent of the total number per litter. Resorptions, dead, stunted, and malformed fetuses were scored as anomalous. Examples of these types of anomalous fetuses are shown in Figures 5 and 6. Such effects are distinct from immediate effects of microwave radiation on the fetus. Figure 7 shows two

Fig. 7. Appearance of two mouse fetuses at day 15 of gestation after microwave irradiation of the mother. Fetuses were removed within 5 minutes after cessation of exposure to acute sublethal dose. Note hematomas throughout the body, and particularly in the head region.

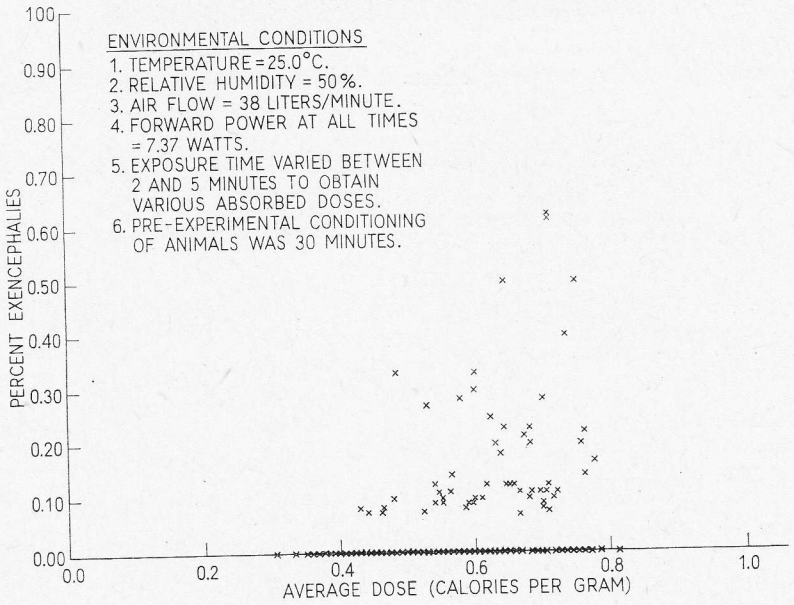

Fig. 8. Incidence of exencephaly in litters exposed to various average doses of microwave radiation. Each point represents the exencephaly in individual litter. Environmental exposure conditions were as indicated on the graph.

fetuses removed at day 15 of gestation, after irradiation at 7.37 watts of forward power (which corresponds to 123 mW/cm²) for less than 5 minutes. The predominant feature is the hematomas, both throughout the body and particularly in the brain region.

Anomalous fetuses were observed through the range of average doses used in the study. Normal litters were also observed almost throughout the same range. However, the number of litters without anomalous fetuses decreased as the average dose increased. The decrease in normal litters suggests the possibility of a dose-effect relationship. However, the available data are too limited and too divergent to establish the relationship conclusively.

Above 4.2 cal/g, exencephalies were observed as shown in Figure 8. Exencephaly

WHO – BIOLOGIC EFFECTS & HEALTH HAZARDS OF MICROWAVE RADIATION 1974

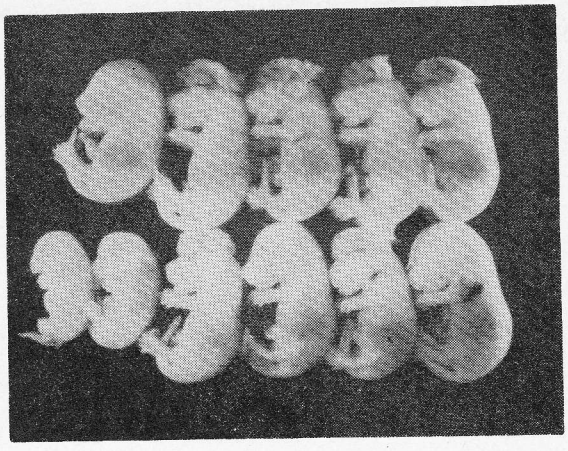

Fig. 9. One entire litter with six exencephalies and two dead fetuses (lower left), and three "apparently" normal. A variety of effects can be observed even within a single litter.

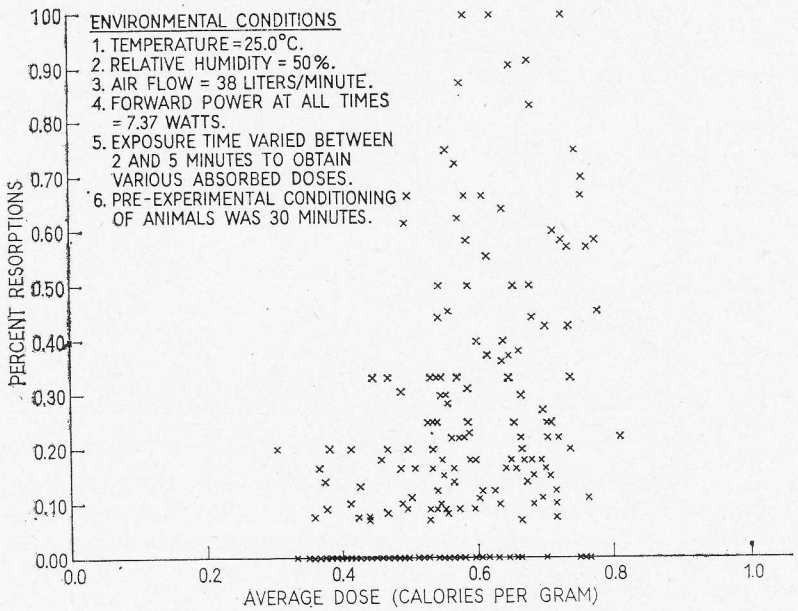

Fig. 10. Incidence of resorption observed after various average doses of microwave radiation. Each point represents the percent of resorbed embryo observed in individual litters.

is a brain hernia which is consistently caused by ionizing radiation in fetuses of the CF_1 mouse. Peak induction of exencephaly by ionizing radiation occurs at day 8 of gestation in these mice. Microwave irradiation did not produce exencephaly as frequently as does x-irradiation (6). The maximum production of the anomaly involved about 60 percent of a single litter. Exencephaly in a single litter is shown in Figure 9. At 8 cal/g of microwave radiation, the average expectancy of exencephaly was approximately 12 percent.

WHO – Biologic effects & health hazards of microwave radiation
1974

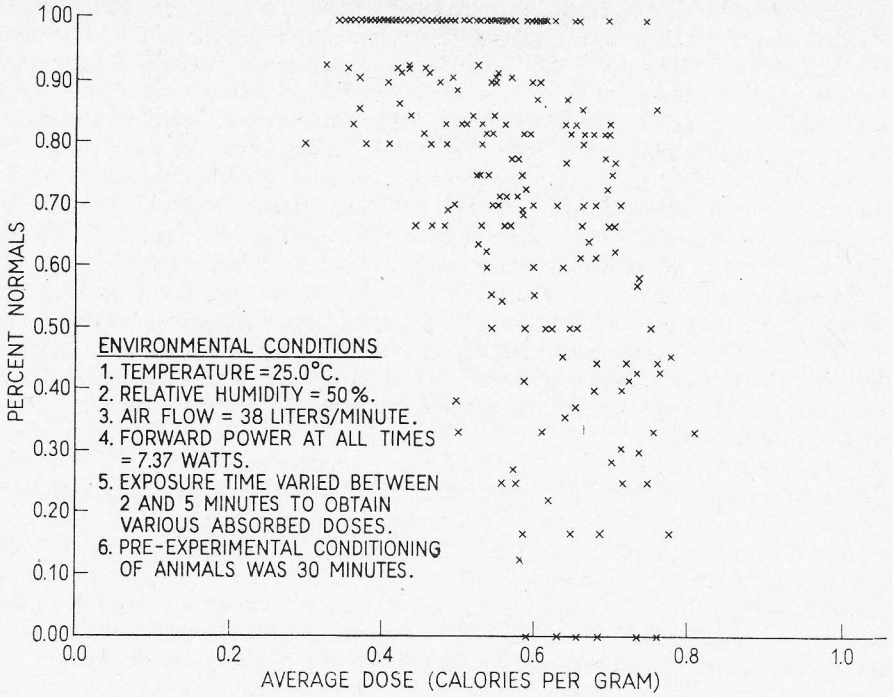

Fig. 11. Incidence of topographically normal fetuses after exposure to various absorbed doses of microwave radiation. Each point represents the percent of "apparently" normal fetuses observed in individual litters.

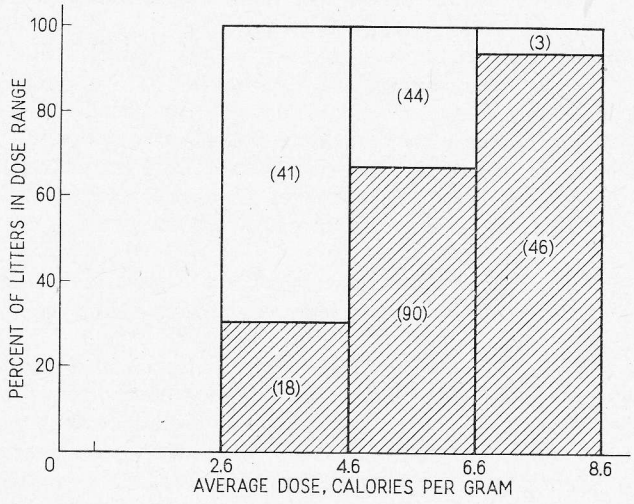

Fig. 12. Frequency of normal and anomalous litters after microwave exposure in the indicated ranges of average dose. Symbols: white columns = all litter mates normal: hatched columns = one or more resorption or anomaly. Total numbers of litters examined are shown in parenthesis.

WHO – Biologic effects & health hazards of microwave radiation
1974

Resorptions are evidence in the dissected uterus that development had begun. The ovum had been fertilized, implanted, and developed to some extent, but development had ceased and there remained only a partially resorbed mass resembling a blood clot. Some anomalies could be included in resorption data, since they could kill *in utero* and certainly the fact of resorption would imply an abnormal condition. Figure 10 shows a very wide distribution of incidence of resorptions, from 3.0 cal/g to 8.2 cal/g. At doses up to 7.8 cal/g, litters were observed in which there were no resorptions. However, at the higher doses the incidence of litters without resorptions is much reduced. Between 6.0 and 8.0 cal/g average dose, three litters were completely resorbed.

The calculation of normal offspring versus average dose is shown in Figure 11. It must be pointed out that normality is based upon rather gross analysis, meaning absence of any easily detectable structural anomaly. Many of the fetuses which were designated as normal could have been abnormal upon histopathological examination. Doses to pregnancies that yielded 100 percent normal offspring ranged from 3.4 cal/g to 7.8 cal/g, with decrease in frequency, while those with 100 percent abnormal offspring were observed beginning at 5.8 cal/g. Only six such pregnancies were produced up to 7.7 cal/g. As can be seen in Figure 12, the frequency of normal litters decreased as the microwave dose was increased. The concomitant increase of litters with one or more anomalous offspring, including resorptions, indicates that a linear relationship may exist between dose and fetal effects. Furthermore, there seems to be no evidence of a threshold effect. It should be pointed out that data were not obtained below doses of about 2.5—3.0 cal/g. The variation of discrete data points, such as seen in Figure 4, indicates that caution is necessary in the interpretation of the incidence of normal and abnormal litters in terms of significance and potential meaning for human health.

CONCLUSIONS AND SUMMARY

The following conclusions have been made on the basis of the study:

1. Microwave radiation can be teratogenic in mouse fetuses exposed at day 8 of gestation to average doses in the range 3—8 cal/g.
2. Gross anomalies observed in microwave-irradiated fetuses include gross hemorrhage, resorptions, exencephaly, stunting and fetal death.
3. Teratogenesis in the microwave-exposed mouse is not an all-or-none phenomenon. There is a dependency between dose and effect, without evidence of a threshold effect.
4. The distribution of microwave dose is unknown, and may account for some of the variability in incidence of fetal anomalies observed. Additional factors relating to the processes by which embryos react to microwave energy must also be taken into account.
5. The fetal anomalies reported in this paper are detectable by direct inspection. One may expect subtle and microscopically observable effects in addition to the gross anomalies.

A waveguide apparatus has been utilized so that integral dose rate to experimental animals can be determined under stable controlled environmental temperature and relative humidity. Pregnant mice were exposed within a duration of 5 minutes to a constant exposure rate of 2450 MHz radiation. Microwave-induced teratogenesis was observed among litters exposed to average doses in the range 3—8 calories per gram. Teratogenic effects observed include gross hemorrhage, exencephaly, stunting, fetal resorptions, and fetal death. Variability in the incidence of anomalies within litters indicates that the cause and effect relationships are not well enough established and understood to permit prediction of effects either in the mouse or in other species.

REFERENCES

1. BELDING, H., HATCH, T. F.: *Heating, Piping, and Air Conditioning,* 1955, **27**, 129.
2. DEICHMANN, W. B.: *Archiv. Toxikologie,* 1966, **22**, 24.
3. HAINES, G. F., JR., HATCH, T. F.: *Heating and Ventilating,* 1952, **49**, 94.
4. HO, H. S., GINNS, E. I., CHRISTMAN, C. L.: Environmentally-controlled waveguide irradiation facility. In: *Symposium issue IEEE G-MTT Transaction,* December 1973 (in press).
5. MUMFORD, M. W.: *Proc. IEEE,* 1969, 57, **2**, 171.
6. RUGH, R.: Neurological Sequellae to Low-Level X-Irradiation of the Developing Embryo. In: *Effects of Ionizing Radiation on the Nervous System.* International Atomic Energy Agency, Vienna, 1962, p. 207.

WHO – BIOLOGIC EFFECTS & HEALTH HAZARDS OF MICROWAVE RADIATION
1974