THE EFFECT OF ULTRASHORT WAVES ON THE RETICULO-ENDOTHELIAL SYSTEM OF THE ALBINO RAT

by

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I

INTRODUCTION

The greater part of the experimental work with animals exposed to ultrashort waves in relationship to histological studies has been done with apparatus which produces such a powerful field as to cause pathological changes in the tissues. In recent years there has been a tendency to avoid long irradiations which are accompanied by a sensation of considerable heat. Clinically the best results have been achieved with frequent irradiations of short duration which produce a minimum feeling of warmth. This is accomplished with higher frequencies of 5 meter wave lengths or less.

The effect of the ultrashort waves is intimately related to the physiological functions of the living body as a whole, therefore in-vitro experiments on isolated tissues are misleading. In reviewing the literature, the primary factor in the results produced with ultrashort waves appears to be the local vasomotor stimulation, resulting in an increased circulation of the blood in that part which is exposed to the high frequency field. If it can be demonstrated that the reticulo-endothelial system is also stimulated to increased activity, some of the therapeutic effects of high frequency currents might prove to be attributable to another definite physiological process.

II

ULTRASHORT WAVES

Review of the Literature

The therapeutic use of ultrashort waves developed from the basis of Clerk Maxwell's mathematical theory that light and electromagnetism were related. This relationship implied that electromagnetic waves could be sent through space in a manner similar to light rays, and thus this theory became the basis of radio transmission.

Hertz, in 1888, was the first to obtain a wireless transmission. Using an induction coil as a sending apparatus, he was able to pick up electromagnetic radiations by a loop of wire held some distance away in his hand, using the air as a connecting medium. These waves became known as Hertzian waves and the term is interchangeable with the term radio waves.

Marconi, in 1901, repeated this experiment on a much larger scale, transmitting the letter "S" across the Atlantic Ocean. Along with the wireless came the radio, the wavelengths used ranging from 30,000 meters to about 5 meters. The longer wavelengths were first known and used, and when this band of frequency became too crowded, the amateur radio broadcasting stations were given the little-known shorter wave lengths. Frequencies of 20 meters were designated as short waves and those below ten meters as ultrashort waves.

The frequency of a wave is determined by dividing the velocity of light waves (300 million meters or 186,000 miles per second) by the length in meters of the wave. As the wave length becomes shorter, the frequency of oscillation becomes more rapid.

In relation to other wave lengths in the spectrum, ultrashort waves are extremely long. The infra-red ray has a wave length of 0.02 centimeters and first visible light rays have a frequency of 400 million million cycles per second. Ultrashort waves are immeasurably longer than the gamma and X-rays. Conrad Cale[1] compared the eye with a receiving apparatus which is tuned to a band of high frequencies between 400 and 750 million kilocycles, registering colors from red to violet and not responding to frequencies either above or below this range.

The use of high frequency waves therapeutically was first suggested by Tesla in 1891, and during the same year, d'Arsonval experimented with high frequencies for medical purposes. In 1920 W. R. Whitney observed that men working with a powerful short wave transmitter equipment in the research laboratory of the General Electric Company had a rise in body temperature. It was also noted that solutions of electrolytes placed in the electrostatic field of the oscillator did not heat alike, varying in relation to the concentration and the frequency of the field. This led to investigations of the heating of electrolytes in high frequency fields and the use of these fields as a means of producing artificial fever for therapeutic purposes in human beings.

Since about 1926 an increasing amount of research has been done with ultrashort waves. There are two aspects of the problem around which most of the experiments have been directed, and which have caused some controversy. The effects of high frequency currents have been ascribed to two causes, namely, heat effects, and secondly, specific biological effects which cannot be fully explained as due to heat. Some investigators have concluded from their experimental work that

there are specific effects due to causes other than heat. The amount of evidence confirming these two theories is more or less balanced. The specificity of the wave length is another factor which is little understood.

With alternating currents (such as those used in diathermy and in short waves) displacement and conductive currents must be considered. A displacement current is the distortion of the orbit of the electron within the atom. A conductive current is a drift of electrons from atom to atom along the path of least resistance. All substances permit the passage of current, either the conductive or the displacement current, at high frequencies.[2] As the frequency becomes higher there is an increase in the passage by displacement or capacity current. A capacity current is the result of the resistance of electrons to displacement.

The heat effect produced in the tissues by a high frequency field is due to the displacement of electrons within the atoms or molecules composing the tissues. This displacement may theoretically consist of an orbital distortion of the electrons in the case of non-conductors or a drift of electrons from atom to atom in the case of better conductors. In either instance there is an energy loss in the form of heat. The body placed in a condenser field acts not as an Ohmic resistance but as an additional dielectric, thus enabling a definite amount of heat to be introduced into every depth.

According to Nahlisphake[3] there are four essential properties inherent in ultrashort waves: (1) A special thermal action, which directly effects the smallest particle, (2) a localised depth effect, (3) a selective thermal action, (4) a specific effect on colloids.

The capacity current passing into the tissues produces a state of hysteresis. The disruptive blows or impacts of electromagnetic vibrations oscillating at a very high frequency act upon the smallest

particles of a coil. As mentioned above, one of the results of this impact is the conversion of the arrested kinetic energy into heat. The dynamic, pounding action must be more in evidence in the capacity currents than when the current flows freely through a good conducting medium. The electromagnetic stresses must be greater in the former case.

Schliephake[4] offers the theory that the ions are subject to a power drag in the direction of the field. In a high frequency field the direction of the field is changed with the oscillations, thus causing the ions to be moved very rapidly to and fro by the electric field force. In this way a conduction current is produced and thus heating of the dielectric is brought about. Schliephake believes that there is experimental proof that ultrashort waves may produce effects which are not to be regarded as heat in the common sense of the word. Heat is irregular motion of all molecules in a warmed area, but in the short wave field there is regulated motion of definite particles and groups, and these are dependent on wave length.

Esau's water and oil experiment has become classical because it demonstrates the selective action of short wave radiation upon small particles of matter. When alkalinized water is mixed with paraffin oil and this emulsion is thoroughly shaken and placed within a short wave field, there soon follows a boiling effect with an escape of steam. Temperature measurements of the liquid at that time show that it rose only 60 to 70 degrees C. while the temperature of the rest of the mixture remained considerably under the boiling point.

Since living tissue is made up of electrolytes in solution it is of practical value to know that selective frequencies have been found to have a specific heating effect on different dilutions of electrolytes. Melonman and Burton[5] and Marshall[6] found that the heating of a solution of an electrolyte in a high frequency field does not depend upon the

composition, but on the specific conductivity and concentration of the liquid and rises to a maximum for a certain conductivity no matter what the size or shape of the specimen heated. Richards and Loomis[7] found that the conductivity at which the maximum effect occurs is proportional to the frequency. For instance at lower frequencies the maximum heating is observed in solutions of lower concentrations than at higher frequencies. According to Pitzold, every dilution of an electrolyte has its specific or maximum wavelength, located between 15 and 2 meters.

According to the work of Bachem[9], for a 5 meter wave length capacity field, the relative heating of excised tissue in descending order is: fat, bone marrow, bone, lung, skin, spleen, liver, hair, brain and muscle. Blood was found to be heated about one-half as much as skin. Blood corpuscles were heated more, blood serum was heated less than whole blood.

Schliephake[10] worked with tissues in vivo. His graphs show that while the subcutaneous structure attains the lowest temperature, the curves show that heating is first most prominently located in the muscle, the temperature rising to a high, plateau-like curve and then sharply falling, while bone, which is heated up less rapidly, rises to a higher level than the muscle, and retains the heat for a longer period.

Selective effects have been demonstrated on the constituents of the blood. McLennan[11] showed that shorter wave lengths produce greater heating effects on the corpuscles of the whole blood. Schliephake[12] experimented with equal quantities of whole blood, serum and clots from the same blood exposed to a field of 5 meters for three minutes. The temperature of the whole blood rose to 57 degrees C., the serum to about 47 degrees and the clots to about 52.7 degrees C. From this it would appear that the red corpuscles even in the whole blood become

heated more strongly than the serum in which they are suspended. According to Schaefer[13] also, the absorption of electrical energy by erythrocytes from a 3 meter condenser field is greater than by the surrounding serum.

With ultrashort waves it has been possible to obtain inhibitory or destructive action in vitro and vivo on species of pathogenic bacteria and fungi. It has been found to be necessary, however to work with a definite wave length, because the action of the ultrashort waves upon micro-organisms is selective. The maximum effect is obtained for each species irradiated with waves of specific length. With other wave lengths there are no effects or the effects may be directly opposite in that they stimulate the growth of the organisms.

Liebesny and his collaborators[14] have demonstrated a peculiar selectivity of waves of 15 and of 4 meters on many cocci, bacilli and fungi. These reports have been confirmed by Groag and Tomberg. Isar and Pamilari[15] also found no effects or inhibitory effects on various pathogenic bacilli and cocci according to the length of the wave used.

The time required for lethal action of different wave lengths on Staphylococcus autoanalyticus albus was studied by Schliephake and Haase[16]. The cultures were placed in a condenser field at a temperature maintained precisely at 55 degrees C. The lethal time varied from six minutes to fifteen minutes, according to wave length. The controls were destroyed in the water bath at 55 degrees C. after thirty minutes. These investigators showed that selectivity and specificity are not limited to one wave length, but are scattered over a wide range in which radiation at 20 meters wave length appeared to have the greatest lethal effect, and at 6 meters the lowest effect. These studies dealt with a range of Hertzian waves up to 100 meters.

Daussat and Dognon[17] also have found that bacteria react to

short wave condenser fields in an individual manner for each species. Koch's bacilli have been destroyed by a wave length of 4.8 meters and streptococci by 3.6 meters.

A number of investigators, however, have obtained negative results. Gale[18] subjected Staphylococcus aureus, streptococcus and typhoid organisms to high frequency currents. They were irradiated in a water bath to keep a constant temperature of about 36 degrees C. The duration of exposures varied from 15 to 60 minutes. In some cases the exposures were repeated as many as three times. He found that the bacteria were not destroyed.

As a result of experiments carried out in vivo, Kidinow[19] concluded that ultrashort waves have no specific action on bacteria. He sealed pieces of gauze with bacteria and placed them under the skin of animals and exposed them to wave lengths of 3.4 meters. The bacteria were undamaged with doses of these rays which killed the animals.

Kling[20] found that ultrashort waves had no influence on the course of tuberculous infections in guinea pigs.

These contradictory results are perhaps due in part to the fact that the lethal wave length is specific for different species and strains of bacteria.

The relatively great surface area of bacteria indicates a high metabolic rate. The stress and strain produced in the molecules of the organism by the displacement current would tend to increase the rate of metabolism. The degree of increase could be slight and thus stimulate the growth and reproductive processes, or it could be sufficiently great to cause the death of the organism. It is possible that the specificity of the wave length may be related to the individual rates of metabolism of different species and strains of bacteria.

Therapeutic attempts with short waves were suggested to Izar and Moretti[21] by experiences gained with the germicidal effect of short waves upon some strains of Brucella hominis. The bacteria were destroyed by 4 meter and 8 meter waves while 16 meter waves did not show any such effect. Nine cases of Malta fever were treated with ultrashort waves. Six of the nine cases were benefited. Two of the cases gave negative results. Duration of the treatment was from 15 to 20 minutes with daily exposures or at intervals of a few days. These authors believe there is a certain relationship of the mechanism of short waves to vaccinotherapy and therapeutic fever.

Carpenter and Bonk[22] reported that high frequency irradiation of 30 meter wave lengths will cause elevations of temperature and prevent the development of experimental scrotal chancres in rabbits.

The effects of ultrashort waves on serological reactions have also been studied. Izar[23] showed that a 20 minute exposure to wave lengths of 4, 8, and 16 meters increased the non-specific anti-complimentary power of normal and syphilitic serum, the increase varying inversely to the wave length. Izar and his collaborators obtained positive and negative results in producing modifications in the formation of agglutinins and precipitins according to the length of the waves employed.

Phisalix and Pasteur[24], using waves of 20 meters, destroyed the anti-venom substance and the neuro-toxin of the venom of the viper, while the hemorrhagic substance remained unmodified.

Szymanowski and Hicks[25] found that ultrahigh frequency currents from 1.2 to 3.7 meters were capable of producing definite attenuation of three major bacterial toxins, diphtheria, tetanus and botulinus, in raw broth filtrates. This effect was obtained without the

development in the toxin of temperatures that would by themselves affect the potency of the toxin. These authors advanced a tentative theory as follows: Since toxins are large molecular complexes and the bonds connecting the components may be rather weak, because a comparatively small increase in molecular agitation owing to elevation of temperature is able to destroy them in part, it is possible that a similar partial destruction occurs as the result of the rapid agitation of the molecular dipoles in the high frequency field and the resistance opposed to this motion by the viscosity of the fluid.

The work just mentioned above confirmed the experiments of d'Arsonval and Charrin[26] in 1896. These workers found that high frequency currents of 300,000 cycles per second (wave length of 1,500 meters) would diminish the strength of diphtheria toxin. This effect was produced without an elevation of temperature which would by itself affect the toxin. It was further indicated at that time that the toxins attenuated by irradiation had special immunising properties.

A considerable amount of experimental work on the constituents and the circulation of the blood has been done with ultrashort waves.

It has been found that short waves produce a dilatation of capillaries, small arteries and veins, the same as any other heat stimulus. This dilation produces an acceleration of the blood stream and results in an active hyperemia. Intense or prolonged application of short waves has been shown, in the web of the frog's foot, to result in injury to the circulation. If the capillaries are dilated to an excessive degree there appears at first a slowing and finally a complete arrest of the normal path of the circulation. There sometimes follows a return flow from the veins to the capillaries. This excessive dilation continues to exist for a number of days (up to 14). The ves-

sels do not contract after injection of adrenalin or on faradic stimulation.

In addition to vasodilatation, blood clotting also becomes so markedly increased that even the addition of sodium citrate has no appreciable influence in retarding it. This is in accord with Kobac's[27] observation that short wave radiation markedly reduces the clotting time of blood.

It has been accepted that the apparent paralysis of the vessel walls results from some effect of the short waves on the sympathetic nervous system, producing greatly reduced tonus, and even temporary paralysis. All of these findings can be modified and even controlled when the dosage is kept within moderate limits.

The hydrogen ion concentration of the blood is increased by irradiations of ultrashort waves. Change in clotting time can perhaps best be explained by the effect of the short waves on the calcium ions. These ions are reduced in the blood but increased in the tissues.

The serum of normal individuals viewed in a dark field showed an enormous number of actively motile protein particles. In health the particles are relatively small and in constant brownian motion. In disease the motion is more sluggish or totally arrested. The particles either are diluted out by lysis, become anaironic in size, or tend to clump, precipitate or assume giant size in the form of rings. Precipitation is a fairly constant phenomenon of the initial stage of short wave treatment and dispersion the eventual effect.

Experimental data[28] show that in hyperthermia produced by high frequency electric current, the blood was concentrated as much as 40 per cent or more. Below a body temperature of 41 degrees C. the hemoglobin saturation of venous blood was increased; above this

temperature it was diminished. The loss of water from the blood stream was accompanied and probably partially compensated for by a large storage of corpuscles in the spleen. The carbon dioxide content of venous blood was reduced as much as 30 per cent. The alkali reserve, as measured by carbon dioxide capacity, was decreased, but to a lesser extent than the carbon dioxide content. It is suggested that this disturbance in the acid-base relations of blood is a consequence of three factors: Hyperthermia, hyperventilation, and a greatly accelerated metabolic rate.

Knudsen and Schaible[29] exposed dogs to an ultrahigh frequency field and studied the physiological and biochemical changes of the blood. They found as much as a 25 per cent decrease in blood volume. The blood cell and hemoglobin concentration returned to normal within 24 hours. A temperature of 41.7 degrees C. does not produce any change in pH, although there is a tendency toward acidosis caused by the great increase of lactic acid. Non-protein nitrogen of the blood increased in some instances to 200 per cent. There was an increase in blood sugar.

These workers found an increase in both red blood cells and total white cells. Besides the increase in red cells there was, in many instances, a marked increase in immature forms of red cells, suggesting a stimulation of hemopoetic tissues. They found an absolute and relative increase of polymorphonuclear leucocytes. The lymphocytes and eosinophils are usually relatively markedly decreased. There is a less marked and constant change in the number of monocytes.

Cignolini and Olivvieri[30] observed the capillaries in the interdigital webs and mesenteries of frogs placed under a microscope in a short wave field. Depending upon the strength of the current

employed, they found with small doses, an acceleration of blood flow and a slight dilatation of the capillaries and arterioles and venules. With moderate doses there was a constriction of the capillaries and arterioles and a slowing of the capillary and venous current. With stronger irradiation, there was ischemia with almost complete occlusion. With very great output, they observed a transient vasoconstriction, then vasodilation, acceleration of blood flow, then finally a slackening, ending in stasis.

Migration of polymorphiles is stimulated and the phagocytic index rises markedly. Jorns[31] favors moderate dosage for increased phagocytosis, as he claims that heavy and prolonged dosage diminishes phagocytosis. McCutcheon[32] studied the effect of temperature upon the motion of leucocytes in vitro. The velocity of locomotion shown by neutrophilic polymorphs of one individual is found to be doubled in vitro by a rise in temperature of 10 degrees C, within certain limits of temperature variation.

Pflaum[33] showed that vascular and vagotonic structures remain affected long after treatment has been stopped. The web of a frog's foot showed marked dilatation of the capillaries and of the larger arterial and venous branches with acceleration of the blood stream and a marked increase in defensive cellular elements.

Some work has been done with ultrashort waves on neoplastic tissues. Scherreschewsky[34] found what he concluded was the most lethal frequency and experimented with transplantable tumors on mice and fowls using frequencies of 66 million to 68 million cycles per second (4.5 to 5 meters). Curative results in the mice were obtained in 25 per cent of the animals, and 50 per cent in the exper-

ments with the fowls. Later he used higher frequencies of 90 to 100 million cycles per second with much better results, obtaining 60 to 75 per cent of recoveries. As to specific biological effects, Scherescheweky treated the tumors with hot water circulating through a hollow copper applicator until the temperature equaled that raised with the ultrashort waves. He found that it was possible to bring about a recession of the tumors, however it required more time for the rise in temperature. He concluded that it was evident, therefore, that the curative effects noted were due to the heating of tumor cells in the high frequency field.

In 1929 Christie and Loomis[35] repeated experiments on mice similar to that of Scherescheweky using frequencies of 150 million (2 meters) to 7 million (43 meters) cycles per second. They found that up to 6 meters (50 million cycles per second) the lethality of the field to be in proportion to its intensity, one frequency being just as lethal as another. Above 6 meters the lethal effects seemed to dimish.

Microscopic sections[36] made of tumors removed immediately and 24 hours after exposure in situ for a space of 3½ minutes at a frequency of 68 million cycles per second, strongly indicated that tumor cells and especially their nuclei, bore the brunt of the attack, the surrounding areolar tissue being much less affected. Fragmentation of the nucleus, disappearance of cell outlines, and pyknotic nuclei of tumor cells were some of the effects noted.

Hoffe Jr.[37] experimented on the action of ultrashort waves of 2 to 3 meters on in vitro cultures of spindle cell sarcoma of the rat and used for a control an in vitro culture of embryonic chick heart. The neoplastic cells were inhibited in their development

much more when the wave was shorter. Spindle cell sarcomas which were previously irradiated were endowed greatly in their capacity to grow and to be grafted on rats. Roffo Jr. obtained similar results by irradiating adenocarcinomas. Normal tissues also were found to be accelerated in their growth.

Dickens, Stanley and Weil-Malherbe[38] studied the action of ultrashort waves of 3.4 meters and 7.2 meters wave length on the metabolism and growth of tumor tissue in vitro. No effect on metabolism was observed after the tissue had been exposed to an intense field during one to two hours. After exposure in vitro tumors showed no inhibition of growth when transplanted into animals. They found that tumor tissue in vitro is not more susceptible to heat than normal tissue.

Rondoni and Mezzadroli[39], using waves of 3 meters, found constant acceleration of the rate of growth of adenocarcinomas grafted in white mice.

Gosset and collaborators[40] experimented with tumors of Bacterium tumefaciens in geraniums. This paper, published in 1924, was apparently the first on the biological effects of high frequency irradiations. Using a frequency of 150,000,000 cycles per second (6 meters), they reported that the tumors in the experimental plants became necrotic and easily detachable, while the tumors in the control plants grew to great size. There was a necrosis in the tumor and in the branch which carried it. Rivera confirmed these findings with waves of 2.3 meters if the tumor had been already formed for four months. But when Rivera and Tagenocozzo[41] caused the same waves to act on fresh inoculations of B. tumefaciens the proliferation was stimulated. Rivera interpreted the necrosis in the former instance not as a

depressive effect, but as a stimulation produced by the ultrashort on the tissue. The nutritional needs were increased by the raised rate of cell multiplication and thus exhausted the branch upon which it was found.

Because of these opposing results, nothing should be inferred as to the eventual action of these waves on spontaneous tumors.

The literature also contains descriptions of the effects of ultrashort waves on plants. Beredetti[42] obtained a favorable action on the seeds of maize, grain, barley and rice, which is manifested by a precocity of the plants in the first weeks of growth, with specific conditions of intensity and frequency. For each of these grains he found that a specific wave length gave the maximum stimulation. In some cases the length of the wave which favored the ontogenesis of one species was detrimental to another.

Mesnadrali and Vareton[43] demonstrated the stimulating action of Hertzian waves of 2 to 3 meters on the development and growth of plants and animals. Such wave lengths favored the germination of barley, beans, peas, maize and the increased growth of the respective young plants. They also increased the development of saccharomycetes and therefore their fermenting action. These investigators found that with excessive dosage or duration the influence was detrimental.

Benedetti observed modifications in the division of yeast, and a stimulating or depressing action on the yeast according to the duration of the irradiation and the length of the waves used.

American authors have caused the death of insects and their larvae and pupae with Hertzian waves of medium length which did not destroy the power of germination of the seeds. A practical result

of this observation would be the possibility of freeing the seed of parasitic infestation. Castaldi has shown that the lethal effects on insects after a period of irradiation is less in the species which have a more highly differentiated nervous system.

Malov[44] investigated the death of the Drosophila in the electric field of short and ultrashort waves (from 4.3 to 107.5 meters). He showed that the death of the fruit flies was due to ordinary heating effects and that there were no noticeable differences in effect between short and ultrashort waves.

A variety of interesting results have been observed in animal experimentation. A few of these will be noted here. With waves of 2.8 meters Mezzadroli and Varetom[45] accelerated and quantitatively augmented the ontogenetic processes of silk worms. The results differed according to whether the treatment of the silk worm eggs was applied immediately after being laid or a few days after. Irradiating the eggs either before or after incubation produced accelerated development. These investigators found an increase in the weight of the larvae and of the cocoons.

Wetzel and Kiesselbach[46] used 12 and 3 meter wave lengths in studying the effect of ultrashort waves on tadpoles. Heat effect was excluded and they were radiated twice daily for a period of one-half hour. No differences were noted as compared with control tadpoles which were not in the ultrashort wave field.

Nordier[47] observed selective heating and lethal effects on fish irradiated with short waves. The water and the glass tank remained relatively cool as compared with the high temperature in the fish.

Jellinek[48] in his studies of the biological effect of ultra-

short waves chose experiments, methods and conditions which almost totally excluded the heating effect of these waves. Almost all of the experiments were carried out with a very weak field of one watt and nearly always with a 3 meter wave length. In case of chrysalises the development of butterflies took place three to four weeks earlier than in the case of the controls. The incubation period of parrot eggs was not shortened, but the process of maturing took place at an inner temperature of 28 to 29 degrees C. whereas the temperature necessary in an ordinary incubator is 38 degrees C.

New born mice became paralyzed in the ultrashort wave field, but moved again quite naturally when taken out of the field. The same effect can be produced acoustically on new born mice by handclapping.

Jellinek also obtained an increase in the weight of new born mice. Also the hair of these animals became thicker and whiter than in the controls.

Book, Carpenter and Warren[49] exposed male and female rabbits for many hours to irradiation of waves of 30 meters fractioned in several weeks. Not only did it not retard their growth, their fertility and the intra-uterine development of the young, but there resulted in the majority of cases a gain in weight greater than in the controls.

Knudson and Schaible[50] exposed young rats to an ultrahigh frequency field for periods of from one half to one hour daily and raised the body temperature to 40.6 degrees C. This treatment did not seem to retard their growth appreciably. The reproductive organs in the male and female rats were not appreciably affected, so that there was not a loss in power to breed. Repeated exposure produced no abnormal pathological lesions.

A number of histological studies have been made to determine the the tissue reactions to irradiations with ultrashort waves. Experiments in this field, in most cases, used extreme dosages, consequently the findings have shown some pathological changes.

Jacobson and Hosoi[51] using waves of 25 meters, treated dogs, guinea pigs and white rats from 37 minutes to 12 hours. The histological changes noted were hyperemia, dehydration, cloudy swelling, fatty changes and focal hemorrhages. Lymphoid tissue showed much stimulation, and with longer, continued heating, necrosis of the germinal centers. Occasionally necrosis in the crypts of gastric and intestinal glands with leukocytic reaction was found. Kidneys, liver, and heart usually showed marked fatty degeneration. In the liver, the changes usually began in the peripheral zone of the lobule. The ovaries were normal except for hyperemia. The testes showed marked edema, congestion, occasionally degenerative changes in spermatogenia and spermatids, with proliferation of Sertoli cells and formation of giant cells free in the lumen of the tubules. The brain showed hyperemia, edema and subpial hemorrhages. Scattered intercortical hemorrhages were rather constant and also chromatolysis of the nerve cells of the pyramidal layer. Glycogen depletion of the liver was moderate in rats heated for short periods and complete after four hours of heating. Bone marrow was hyperactive in all of the rats.

Saidman, Myer and Cohen[52] placed rats in an oscillating circuit with a frequency of 30 million cycles per second. The head, thorax and abdomen were irradiated separately. Irradiation of the head caused heightened temperature of this region, exophthalmia and then death. Autopsy revealed meningeal congestion and fatal congestion of the lungs and cardial dilatation. The temperature of the abdomen was raised

by irradiation and exophthalmia was produced. Autopsies showed the action to be a selective one on the blood, causing death by embolism. Fresh human blood injected intraperitoneally in the rat was coagulated upon irradiating the animal.

Schliephake[53], collaborating with Osterteg and Strassburger obtained experimentally selective changes in animal cells. By treatment of the brain and medulla oblongata of rabbits peculiar disturbances of the regulation of temperature were produced. Histologic examinations made by Osterteg showed selective changes of certain kinds of ganglionic cells of the dorsal vagal nucleus. Other cells of a somewhat different kind but close by, remained unharmed. This type of damage could be produced only with wave lengths below 3.5 meters.

Horn, Kaudere, and Liebesny[54] used 15 meter wave lengths on 10 schizophrenic patients, avoiding any appreciable heating effect. In each case, 30 exposures of 20 minutes each were given. In several cases slight improvements were noted such as a soothing influence and an increase in interest on the part of the patient. Examination of the spinal fluid revealed an increase of the total proteins, and transiently an increase in cell number. To explain these changes, animal experimentation was resorted to. Autopsy of the animals showed that the action of the ultrashort waves selectively attacked the vessels and the meninges, the vessels of the latter being especially affected. Change of cerebrospinal fluid was caused by injury of the vascular system. The factors responsible appeared to be permeability of the meningeal vessels and especially direct passage of constituents of the blood into the space containing the cerebrospinal fluid, as caused by rupture of vessels.

The only work that was found described in the literature which was directly related to the problem of this thesis was that of Somad[55]. After intravenous injection of trypan blue, diathermy was applied bi-temporally to the heads of rabbits for ten to fifteen minutes with moderate dosage. In comparison with controls, there was an increase in dye in the choroid plexus and in histiocytes. In the epithelial cells it was present in droplets between the nucleus and the periphery, not as fine particles. Somad concluded that diathermy very probably increased the permeability of the plexus portion of the blood-spinal fluid barrier as seen from colored spinal fluid in moderate staining. The concentration of the dye however, remained at a minimum so that no signs of nervous irritation appeared.

There is an increasing amount of literature dealing with the clinical aspects of ultrashort waves. Only some theories that have been advanced will be mentioned in this paper. Justina Wilson[56], in discussing some recent advances in medical electricity stated that short wave therapy is the nearest approach to true or corrected metabolism which can be brought about only through cellular or tissue stimulation. In further discussion, she states that the first effect of hyperemia caused by the ultrashort waves is relief of pain and reduced temperature and swelling. Pflum attributes this partly to heat and partly to inhibitory effects on the sympathetic tone and to stimulation of the vagus. Hyper-leucocytosis and increased phagocytosis have an attenuating effect on the virulence of invading bacteria. This would raise both local and general resistance.

Bauwens[57] postulates that non-specific defensive processes are elicited and that some intermediate substance may possibly be liberated.

It follows that the bactericidal action of this therapeutic agent resolves itself into an indirect one due to the defensive processes initiated in the tissue.

Wolf[58] in discussing the physiological basis of short wave therapy says that the defense mechanism is situated to some extent in the fixed cells but probably to an even larger degree in the blood serum and the white cells. If this is the case it becomes obvious that a good circulation is of the greatest importance in a curative process. In tissues in which the circulation is seriously impaired, bacteria may freely develop and cause infectious processes, while a normal circulation may facilitate the destruction of microorganisms by strengthening of the defensive mechanisms. Pflaum's experiments with capillaries of a frog's web substantiate this.

Aside from the influence on the defensive mechanism the improved circulation has also a marked effect on the sensation of pain. Gaza and Brandi, Hübler and Hummel, and others have shown that the subcutaneous and intramuscular injection of substances with a pH less than seven produces sever pain which becomes unbearable when the pH of the substance injected becomes 5.7. Inflammatory tissue has a low pH and thus the inflammatory edema causes so much pain while the allergic edema has a normal pH and is not painful. Short waves dilate the capillaries, improve the local circulation, increase the supply of alkaline blood, reduces the acidity of the tissues and thus relieves the pain on irritation and hastens recovery.

Stiebock[59] judging from his experience with short wave therapy over a long period of time considers that there is no advantage in attaining a noticeable heating effect. He stresses that the best results are attainable by diminishing as much as possible the period of radia-

tion and by increasing the number of applications. Accordingly the time of applications should exceed four to five minutes only in exceptional cases. The sensation of warmth experienced at the end of a treatment represents the upper limit of the dose.

III

DISCUSSION OF THE

RETICULO-ENDOTHELIAL SYSTEM

Metschnikoff[1], while studying the connective tissues of Metazoa in 1892, discovered phagocytic cells that play an important role in the defense reactions of the organism. He called these cells macrophages. Ranvier[2], however in 1890, had already given a histological description of similar cells in the omentum of mammals, using the name clasmatocytes. This term was used because in addition to phagocytic properties he observed a pinching off of fragments of the cytoplasm. The appearance of pinched off fragments was probably an artefact due to his special technique in which he used osmic acid fixation, since his observations have not been confirmed in tissue cultures of these cells.

Marchand[3] in 1900 indentified the clasmatocytes of Ranvier with elements which lie along the course of blood vessels and which he called adventitial cells. Maximow[4] in 1902 found similar cells diffused in the connective tissues of mammals and called them polyblasts and resting wandering cells. In the meantime, Mallory's[5] studies on typhoid fever in 1898 had demonstrated that the large phagocytic cells of the inflamed lymphoid tissue developed from the endothelium of the lymph and blood vessels, and thus Mallory termed these cells endothelial phagocytes.

The introduction of vital staining extended the conception of the resting wandering cells. When colloidal solutions such as India ink or one of the azo dyes are injected intraperitoneally, intravenously or subcutaneously into the animal body, the dye particles are electively accumulated by cellular elements which are distributed throughout the entire body. The color of the dye injected can be observed grossly

in the tissues of animals that have been injected with a sufficient amount of the dye. According to the quantity of these cellular elements the different tissues and organs will appear lighter or darker. The ability of certain cells to store colloidal dyes appears to be associated with their properties of mobilisation and amoboid movement and the power of phagocytosis.

Ribbert[6] in 1904 showed that certain cells were specifically stained with lithium carmine and succeeded in demonstrating that these same cells which were vitally stained were able to absorb other substances that were injected, such as iron and lipoids. Bouffard[7] in 1906 was the first to show that these cells could be stained intravitally with the benzidine dyes.

Using Ehrlich's dyes, Goldmann[8] in 1909 demonstrated that the plasmatocytes, adventitial cells, resting wandering cells and adventitial phagocytes observed by the workers mentioned above were identical with the vitally stained cells. Among the workers who followed this line of investigation were Aschoff and his pupils Kiyono and Taschaschin in 1913 and 1914. These investigators expressed the idea that resting wandering cells of loose connective tissue belong to a great cell group that appears in different forms and plays an important role in general metabolism. Kiyono introduced the term histiocyte which means tissue cells.

While studying the metabolism of cholesterol in 1914, Landau and McNee[9] concluded that there is a "reticulo-endothelial metabolic apparatus" in the animal body. Later it was shown that the vitally stained cells were part of this system.

The reticulo-endothelial system according to present conceptions is made up of cells which occur in the common loose or dense, con-

nective tissue and in the serous membranes, especially the omentum. Also the reticular cells of the lymphoid and myeloid tissue and of the red pulp of the spleen are included. Cells of the reticulo-endothelial system are also found in the venous sinuses of the bone marrow and spleen, and as the cells of v. Kupffer in the liver capillaries.

The term "reticulo-endothelial" system is not entirely correct according to the views of Maximow, since the endothelial cells lining the blood and lymph vessels do not form phagocytic wandering cells. They store carbon particles and may form fibroblasts in pathological conditions, but they do not have the power to produce histiocytes. The confusing element which caused the inclusion of the endothelium in this system was the formation of histiocytes from the flattened littoral cells lining the sinuses of the lymph nodes. These cells are not endothelial cells in the true sense of the word but are formed from mesenchymal elements and are true histiocytes.

In the human embryo at stages of from 15 to 20 mm., Evans has observed histiocytes scattered sparsely throughout the general mesenchyme. In the adult the histiocytes develop from lymphocytoid or monocytoid, haematogenous or histogenous wandering elements. These in turn are mobilised from undifferentiated mesenchymal elements, especially the small, inconspicuous reticular cells in the lymphoid tissue and from the littoral cells which line the sinuses of the lymph glands and the spleen.

The relationship of histiocytes and monocytes, leucocytes and lymphocytes and fibroblasts is still unsettled. Cunningham, Sabin, and Dean[11] think that monocytes and histiocytes represent two distinct cell strains. They base their views upon their observations of the reactions of the respective cell types to supra-vital dyes. (Vascular

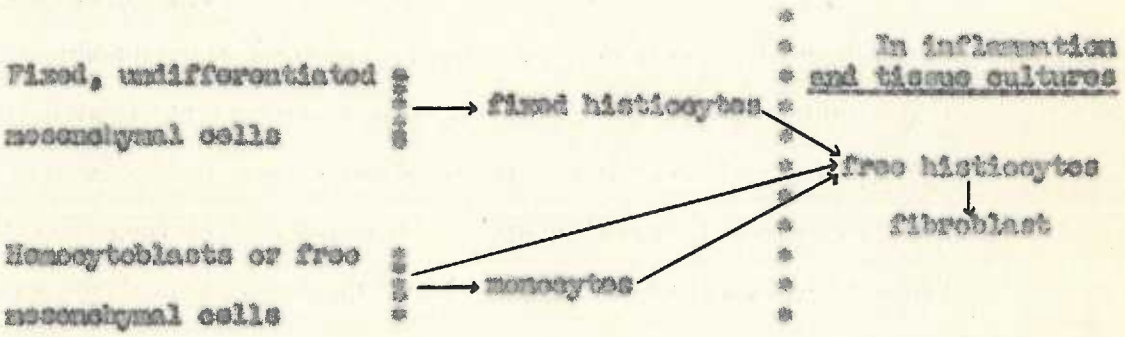
inclusions of the phagocytic cells are stained supra-vitally with dyes such as neutral red in very dilute solution.) According to their interpretations, the monocytes have fine vacuoles arranged in the form of a rosette around a clear centrosphere and these vacuoles stain a fairly constant salmon pink color. Histiocytes on the other hand are characterized by vacuoles of digestion which vary conspicuously in size and color when stained with the same technique. Monocytes are less active as phagocytic cells, but the presence of the rosette limits the position of the engulfed material to the periphery of the cell. These investigators found that the plasmatocytes have the maximum reaction to vital dyes. The particles are large in size and lack a pattern in the cytoplasm.

Maximow[12] and his school however, do not consider the rosette as a specific feature of the monocytes. Maximow believes that active histiocytes originate from local fixed histiocytes and emigrated monocytes and lymphocytes. Aschoff's[13] view agrees in part with that of Maximow, however Aschoff does not hold that the histiocytes originate from emigrated lymphocytes.

By using the technique of tissue culture Lewis and Lewis[14] demonstrated that large mononuclear cells which were incubated in blood became transformed into histiocytes. Lewis[15], using supra-vital staining technique, found transitional stages between large mononuclear blood cells and large macrophages in fascia. These findings are in agreement with the observed transformation of monocytes into macrophages in cultures of blood, and indicate that the same process occurs in the tissues. In this case the question arose as to the origin of the mononuclear cells. Mallory[16] believes that these cells are of endothelial origin. Aschoff and Kiyono[17] maintain that there are three types of large

mononuclear cells in the circulating blood; (1) blood histiocytes arising from reticulo-endothelial cells, (2) transitional cells belonging to the myeloid series, and (3) mononuclear cells of lymphoid origin. Sabin, Doan and Cunningham[18] believe that there are four types of leucocytes in normal blood. These are polymorphonuclear leucocytes, lymphocytes, monocytes and histiocytes. As mentioned above, these investigators were able to discriminate between the mononuclear cells and the histiocytes by supra-vital staining.

The commonly accepted views of the relations between the various cells of the connective tissues may be represented as follows: (Modified from Maximow)[19].



The most characteristic feature of histiocytes is their ability to store acid aniline dyes in their cytoplasm. Colloidal particles of the dye enter the cytoplasm. Here these particles are accumulated in the "segregation apparatus" of the cell.

These "vital dye" granules are neither chemical combinations of the dye with the protoplasm nor physical staining of pre-existing cell organs, but are actual accumulations within the cell of the vital dye in fluid, high colloidal or crystalline form. This theory is in accordance with the conceptions of Evans and Schulemann[20]. The number and size of the dye granules increase with increased dye dosage. By

appropriate dosage a true crystallisation of some of these dyes can be made to occur within the cell. A low dosage will produce macrophages with small vacuoles of strikingly uniform size, while a heavy dosage will produce macrophages with large and small vacuoles.

The "segregation apparatus" is the apparatus by which the dyestuff is separated from the living protoplasm. The macrophages have a much greater power to store vital dyes than is possessed by fibroblasts. The vital dye deposits in the macrophages are however, conversely more susceptible of decolorisation and less permanent than are the more minute deposits in the fibroblasts. (Evans and Scott [21]).

The appearance of histiocytes varies according to their location and their functional state. "Fixed histiocytes" can usually be regarded as phagocytic cells in a resting stage. They appear in many forms, from squamous, round or angular bodies with a spindle-shaped form in profile to irregularly outstretched cells with very long, sometimes branched processes. In the active stage the histiocytes or macrophages appear to be hypertrophied and rounded up into free, amboid cells. In this stage, the nucleus is eccentrically located and kidney-shaped. The cytoplasm is abundant, pale, and slightly basophilic or even acidophilic. In their active condition the histiocytes store larger quantities of vital dyes than they do in the resting stage.

The cells in the reticulo-endothelial system have the ability to phagocytise microscopic particles and to store colloidal, complex chemical substances set free in the body, and thus this system plays an important role in the defense mechanism and metabolism of the body.

In specific infections the reticulo-endothelial system plays a predominant part, for example in the formation of the epithelioid and giant cells in tuberculosis and leprosy. In local inflammatory

processes, the histiocytes act as scavengers and as phagocytic cells to remove bacteria. They also aid in the formation of granulation tissue by removing debris and by transformation of these cells into fibroblasts. In local infections, the degree of resistance depends in a large measure upon the number of histiocytes in the tissues which can be mobilised for active defense. The natural resistance of the common laboratory animals to experimental streptococcus infections can best be explained by the activity of these cells.

The reticulo-endothelial system appears to play a part in antibody formation in the body. By injecting foreign erythrocytes into rabbits, Cary[22] showed that antigenic cells were rapidly removed by the fixed tissue phagocytes of the spleen and liver. He demonstrated by extraction methods that the organs rich in fixed tissue phagocytes were correspondingly rich in specific antibody content.

When the reticulo-endothelial system is blocked with heavy injections of solutions containing colloidal particles such as used in the vital staining technique, it has been observed that the ability of the histiocytes to absorb another substance or to perform a certain function is impaired. For example Gay and Clark[23] found that it was possible to suppress antibody formation (hemolysins and precipitins) almost completely by injections of trypan blue alone. Ross[24], however, finds no support for this observation. Saturation of rabbits by daily intraperitoneal injections of trypan blue for periods up to one month had no effect on subsequent haemolysin formation in these animals in response to intravenous injections of sheep red cells. Jungeblut and Berlot[25] were able to check the production of antitoxin in guinea pigs by large intravenous doses of India ink. Bieling and Isaac[26] found that splenectomy alone in mice had little effect on antibody formation.

but if combined with injections of colloidal iron oxide to eliminate the activity of the remainder of the reticulo-endothelial system, the animals were unable to manufacture hemolysins.

In contradiction to these observations, Rosenthal and Standen-
ath[27] found that vital dye injections increased the antibody concentration. Weiss and Kunze[28] found no change. Lewis and Loomis[29] found that injections of trypan blue increase the capacity of the animal to react to antigenic substances. These investigators remarked that substances which cause physiological activity serve as stimulants in certain doses but become depressants if their action is carried to the extreme.

The histiocytes play an important role in hemoglobin and iron metabolism by phagocytizing worn-out erythrocytes. It is believed that they absorb and transform the hemoglobin. It has also been demonstrated that they play a part in the metabolism and storing of fats and lipoids.

IV

MATERIAL AND METHODS

White rats were used as experimental animals. Pathological conditions caused by too powerful irradiations and of too long duration were avoided. It was attempted to reproduce as nearly as possible the conditions of a clinical treatment. Allowance was made for the comparatively small size of the rat, and the strength of the field was adjusted accordingly.

The apparatus used for this experimental work was a vacuum tube oscillator producing a primary wave of six meter wave length and a rather strong harmonic of 2 meter wave length. The wave length varies several centimeters and cannot be specified exactly for such apparatus because the spacing of the electrodes, the material placed between them, the area exposed, and the objects in the vicinity affect the wave length at such high frequencies. The power output was estimated to be 200 watts, as was indicated by lighting to full candle power of a 200 watt lamp placed between the electrodes. A milliameter in the plate circuit does not indicate the current except very roughly, so that it was considered no more reliable than the brilliancy of the neon indicating lamp provided with the apparatus.

The maximum power output was not used for the small experimental animals. Since one must depend largely on the reaction of the patient with respect to the heat effect produced in the tissues, it was necessary to adjust the strength of the field to the tolerance of the rats, stopping short of signs of discomfort and subsequent burns on the ears or tail.

In order to identify the cells related to the reticulo-endothelial

system more accurately and with greater facility, a vital dye was used. The best results were obtained with intraperitoneal injections of trypan blue, using 2 cc. of a 0.5 per cent solution given every other day for three days.

To irradiate the experimental animals the following procedure was found to be the most adaptable. The rat was rolled firmly in a strip of gauze so that movement was more or less prevented. It was necessary to provide proper ventilation of the body surface so that the animal would not become over-heated and show signs of discomfort. The gauze provided ample circulation of air and at the same time permitted easy observation of the animal.

The electrodes used in the condenser field were thin sheets of copper insulated with rubber. These were fitted into a hollow cylinder of fiber, so that they were approximately as long as a rat's body and about 4 inches in diameter. Precaution was taken so that the tail of the rat did not lie in the high frequency field. This was necessary because the energy tends to concentrate in the dense tissue in this area, causing burns and necrosis.

The duration, intensity, and number of irradiations were varied in the experiments. At the conclusion of each experiment the animal was killed with illuminating gas and the tissues were taken and fixed immediately for microscopic study. The tissues examined were: the fascia from the thigh, axillary and inguinal lymph nodes, spleen and liver. Paraffin sections were made and stained with hematoxylin and eosin and with Maximow's technique in order to demonstrate mitosis and to differentiate the cell types. Safranin was used as a counter-stain with the vital dye.

In sections of the liver of both the control and experimental ani-

mals, the average number of Kupffer cells which contained trypan blue granules was determined by counting these cells in 20 high-power fields taken at random in sections from each animal. The thickness of all the sections was 7.5 microns.

Several experiments were attempted, taking biopsies before and after irradiations with the ultrashort waves. This proved to be unsatisfactory, because the dense tissue of the skin did not offer an adequate representation or study of the reticulo-endothelial system.

V

DESCRIPTION OF EXPERIMENTS

Rats #6 experimental and #6 control

Intraperitoneal injections of 0.5 per cent aqueous solution of trypan blue were given in 2 cc. doses every other day for three days. During this time the experimental animal was irradiated fifteen minutes daily for five days. Irradiations of this duration proved to be too severe, causing burns with subsequent necrosis and sloughing of the distal portion of the tail.

Histological observations of the tissues demonstrated that the inguinal lymph node of the experimental animal contained a larger number of phagocytic cells than the control animal. This quantitative difference was due in part to the increase of the cells of the reticular tissue and the endothelial lining of the sinuses which contained dye granules. In addition to the increase in the number of cells containing the vital dye, there was a difference in the amount of dye in individual cells. The granules of vital dye were of greater size in the histiocytes of the experimental animal, indicating that these cells had taken up the trypan blue more vigorously than those of the control animals. There was no appreciable difference in the number of mitotic figures.

In this experiment the omentum was examined in fresh saline mounts, with the addition of a very dilute neutral red stain. (1-1,000). The cells of the experimental animal appeared to be more active; the state of activity being manifested by a greater number of pseudopodia which suggested more extensive amoboid movement. It is of interest to note that a larger number of cells in the experimental animal were

stained supravitally with the neutral red.

Rats #7 experimental and #7 control

Injections of trypan blue were given as described above. Two irradiations of five minutes duration were given to the experimental animal twice a day for five days during the injections. At the end of this period both animals received three injections of lithium carmine (0.5 per cent aqueous solution) given every other day. For some reason the lithium carmine was difficult to find in any of the tissues examined. This might have been due to the fact that the reticulo-endothelial system was blocked with the trypan blue, thus preventing the phagocytosis of the second dye. When the lithium carmine was evident it appeared in the form of consolidated globules, not in small granules, as the trypan blue. A slightly larger number of these globules were found in the cells of the experimental animal.

Rats #8 experimental and #8 control

Two cubic centimeters of 1 per cent lithium carmine were injected intraperitoneally in these animals. The experimental rat was irradiated on three successive days for five minute periods two and three times a day. No vital dye was found in this experiment in either the experimental or the control animal. The experimental animal showed fewer eosinophils in spread preparations of the fascia than did the control animal. This is in keeping with the results of Knudson and Schaible who found that eosinophils of the blood are usually relatively markedly decreased after exposure to a high frequency field.

The liver of the control animal had a fatty appearance and there-

fore was not studied histologically. The spleen and inguinal lymph node demonstrated no significant difference.

Rats #9 experimental and #9 control

Two injections of 0.5 per cent solution of neutral red were given in the course of three days. The experimental animal was then irradiated on three successive days, in five minute periods three times a day. During the time of the irradiation, two intraperitoneal injections of trypan blue were given.

The inguinal lymph node of the experimental animal showed cells containing neutral red alone in addition to those containing the trypan blue. Most of the phagocytic cells which contained the neutral red alone appeared in clusters around the blood vessels. The control animal showed slightly more numerous cells containing trypan blue, but on closer examination it was found that these cells also contained droplets of neutral red. The lymph node of the control animal did not contain cells showing only the neutral red. This would imply that the ultrashort waves had stimulated the reticulo-endothelial system to produce new cells which phagocytized the dye injected during the period of irradiation. In the control animal, the cells containing the trypan blue also took up the neutral red in addition to the first dye injected.

A greater amount of material from destroyed red cells was evident in the spleen of the experimental animal than in the control. This could be interpreted as an evidence of an increased destruction of red cells. The Kupffer cells of the liver were increased 7 per cent by the irradiations of the ultrashort waves in this experiment as shown by counts of these cells.*

*Note chart, p. 60

Rats #15 and #16 experimental

Rat #18 control

Numbers 15 and 16 received two injections of trypan blue as did the control animal, #18. Rat 15 was irradiated for ten minutes in an intense field. Three hours after this exposure to ultrashort waves the rat was killed and tissues removed and fixed.

The cells of the reticulum in the sinuses of the lymph nodes appeared to be in the process of rounding up to form phagocytic wandering cells. The histiocytes were larger than those of the control and contained more trypan blue. The sinuses contained spaces empty of phagocytes, suggesting that they had been swept out into the circulation, while those of the control were packed with such cells, although few of them contained trypan blue. The germinal centers were clearly evident and the number of mitotic figures were approximately the same as in the control. The liver of the experimental contained 58 per cent more Kupffer cells than that of the control.

Number 16 was irradiated for 10 minutes in an intense field. One hour was allowed to lapse before killing the rat. The liver contained 63 per cent more Kupffer cells than that of the control. The other tissues examined in this experiment did not differ appreciably from those of the control animal.

Rats #19 experimental and #19 control

Both animals received three injections of trypan blue given at intervals of 48 hours. During this time the experimental rat was irradiated for ten minutes every day for six dyas. At the end of this period, both animals were killed and the tissues fixed.

There was very little difference in the number of cells containing

trypan blue in the lymph nodes. Those of the control animal appeared to contain a somewhat larger number. The germinal centers and mitotic figures were more evident in the experimental lymph node.

The liver of the experimental animal contained 71 per cent more Kupffer cells which were more heavily loaded with the vital dye than those in the liver of the control animal.

Rats #20 experimental and #20 control

The same procedure was used as for 19C and 19E. In the inguinal lymph node of the experimental animal, the trypan blue was taken up more extensively and intebively than in the control. The control contained fewer and smaller phagocytic cells in the sinuses.

There was a 70 per cent increase in the number of Kupffer cells in the liver of the experimental rat over that of the control.

Rats #21 experimental and #21 control

The same procedure was used as for the last two experiments described above. Very few macrophages containing trypan blue were evident in the sinuses of the experimental lymph node. In portions of the node the sinuses were practically empty of macrophages. The control lymph node contained more cells stained with the trypan blue. The liver of the irradiated rat contained 111 per cent more Kupffer cells which stained more intensively with the vital dye than did those of the control.

Rats #30 experimental and #30 control

Both animals received intraperitoneal injections of Higgin's India ink consisting of $\frac{1}{2}$ cc. of a 50 per cent dilution in saline.

Another injection of one cubic centimeter of the ink was given in 48 hours. Two days after this, injections of one and two cubic centimeters of trypan blue (1 per cent solution in saline) were given in the same manner. Commencing with the first injection of trypan blue, five minute irradiations were given to the experimental animal twice daily for six days. The control animal received identical treatment with the exception of being exposed to the high frequency field. The animals were killed and the tissues to be examined were fixed in formalin.

One node of the axillary lymph node of the control animal contained a large number of histiocytes heavily loaded with India ink. This result of the injections is unaccountable, for none of the other lymph nodes examined in either experimental or control animal behaved in this manner and being the axillary lymph node, it was not near the point of the injections.

There were a greater number of histiocytes containing granules of trypan blue and a greater number of lymphocytes in the sinuses of both axillary and inguinal lymph nodes of the experimental than in the control animal. The sinuses of the experimental lymph nodes contained fewer cells in the reticulum than did those of the control, the histiocytic reticular cells having rounded up into phagocytic form. These cells of the irradiated animal appeared to have been stimulated to store more trypan blue dye. Examined under the low power lens, the sinuses of the control lymph nodes were stained a pale blue-gray, while the sinuses of the experimental node were stained a bright blue. There were few cells containing carbon particles in the experimental animal and the greater number of these were endothelial cells lining the lymph and blood vessels. The secondary nodes in both control and ex-

perimental animals were in the same state of activity.

In both the liver and the spleen of the control animal, there were more histiocytes containing large particles of carbon than in the experimental animal. However there were 80 per cent more Kupffer cells containing trypan blue alone in the liver of the experimental animal. These observations could be intreprated as an indication that the histiocytes of the experimental animal had been set free in the circulation. The absence of phagocytic cells containing carbon particles form the injected India ink before the irradiation with the ultrashort waves could thus be explained. It also appears that the high frequency field stimulated the formation of new histiocytes to take the place of those containing India ink which had been set free. This conclusion is based on the greater number of cells in the experimental node containing trypan blue granules alone.

Rats #31 experimental and #31 control

The experimental animal was given five minute irradiations twice daily for ten days. On the sixth day both animals were injected intraperitoneally with 1½ cc. trypan blue and again in 48 hours with 2 cc. trypan blue. The animals were killed on the eleventh day.

The histiocytes of the sinuses of the experimental lymph nodes showed very definitely more active storing of the dye granules. The Kupffer cells in the liver of the experimental animal were 25 per cent more numerous than in the control.

Rats #32 experimental and #32 control

The procedure for this experiment was identical to #31. The differences between the irradiated and the non-irradiated animal were less evident. The sinuses of one primary node of the axillary

lymph node of the experimental animal were quite empty of either cells of the reticulum or phagocytes. The granules of trypan blue were stored in somewhat more dense particles in the histiocytes of the experimental animal. The experimental liver contained 15 per cent more Kupffer cells than did that of the control animal.

Rats #32 experimental and #33 control

Both animals received intraperitoneal injections of 1 cc. of neutral red in saline. Another injection of 1½ cc. of this dye was given in 48 hours. Two days after this, injections of 1 and 1½ cc. trypan blue were given in the same manner. Commencing with the first injection of trypan blue, five minute irradiations were given to the experimental animal twice daily for six days. The control animal received identical treatment save that it was not irradiated.

The sinuses of the experimental lymph nodes were filled with pale staining cells which had stored very little trypan blue. The neutral red dye was not evident in either the experimental or control animal. The granules of trypan blue were slightly more evident in the histiocytes of the control lymph nodes. There were 45 per cent more Kupffer cells and indications of greater dye storage in the experimental animal than in the control.

It is of interest to note the functional variation of the cells of the reticulum in the sinuses of the lymph nodes under varying conditions. The rats in experiments #6 to #21 inclusive received rather heavy injections of vital dye. All available cells had rounded up from the reticulum to form active wandering phagocytes containing large globules of trypan blue which almost filled the entire cell. The rats in experiments #30 to #33 inclusive were injected with about

half the quantity of dye. The cells in the sinuses had not rounded up, having remained in the reticular structure, and had stored the dye in fine granules of regular size and shape. This supplies additional evidence of the formation of histiocytes from the reticulum of the lymph node sinuses.

Another observation of some interest was in regard to the reactions of the animals in the high frequency field. There was evidence of increased secretion from glands opening into the mouth. When in the condenser field, the rats would rub their noses vigorously with their front feet, and then continue with the typical licking and cleansing motions. At times this extra secretion could be seen hanging in drops in the hair around the mouth.

Even the very young rats go through the same motions. They also show a scratching motion of the hind limb while in the high frequency field.

VI

SUMMARY AND CONCLUSIONS

White rats were irradiated with ultrashort waves of six meter wave length. The dosage of the irradiations was moderate, stopping short of signs of discomfort and subsequent burns. It was attempted to reproduce as nearly as possible the conditions of a clinical treatment and to eliminate pathological changes in the tissues. The length and intensity of the irradiations were varied with the different experiments.

The cells of the reticulo-endothelial system were studied in microscopic sections in regard to their response to the high frequency field. The tissues examined were: axillary and inguinal lymph nodes, spleen, liver, omentum and fascia from the thigh.

Intra-vital dyes, such as trypan blue, neutral red and India ink are absorbed by the cells of the reticulo-endothelial system. Injections of these vital dyes were given intraperitoneally to aid in determining the possible quantitative and qualitative modifications of the reticulo-endothelial system as a result of the irradiations with the ultrashort waves.

The activity and formation of the cells of the reticulo-endothelial system of the white rat are stimulated by the action of ultrashort waves of a high frequency field. The greatest degree of stimulation was observed following a series of irradiations of moderate duration and intensity over a period of six or seven days.

In view of the phagocytic and storing functions of the reticulo-endothelial system, it can be concluded that moderate and frequent irradiations of ultrashort waves augment the defense reactions and processes of the animal body.

VII

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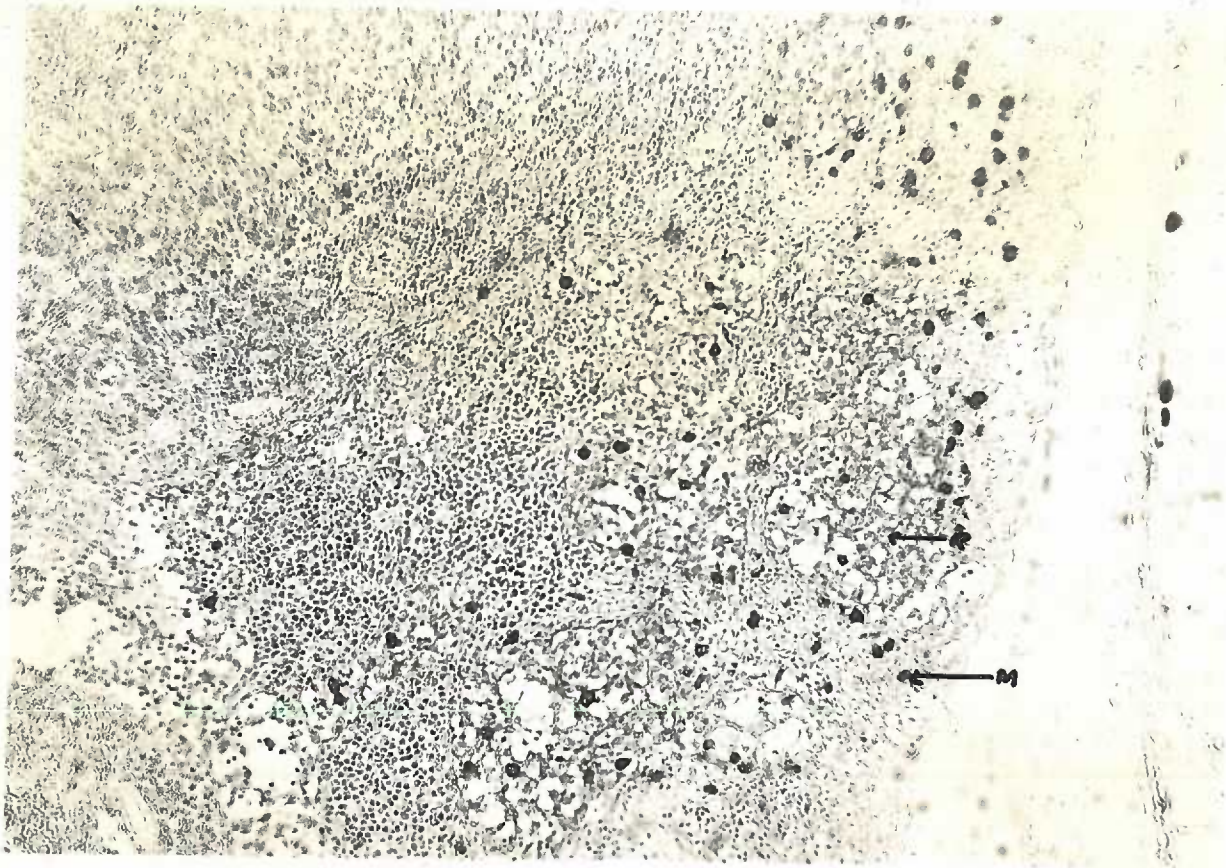
VIII

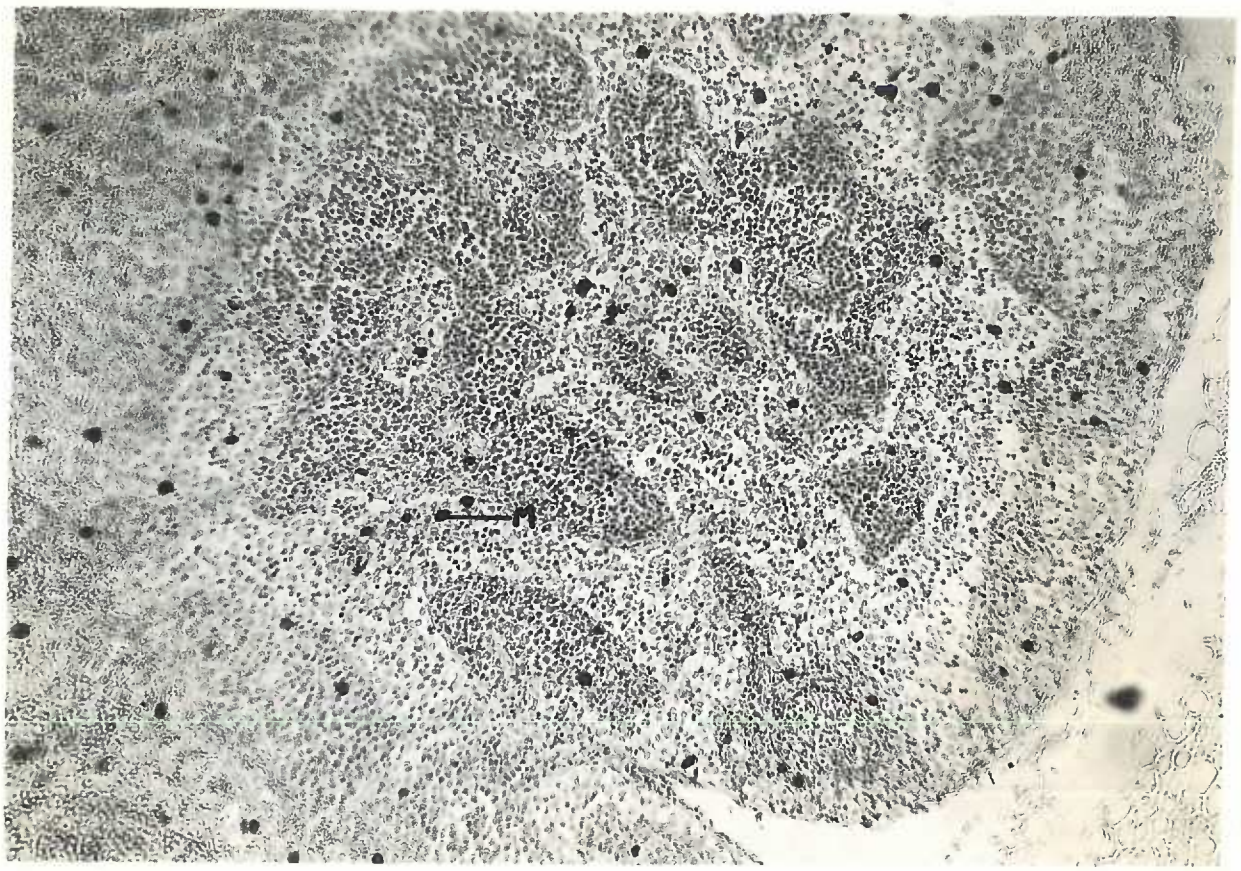
DESCRIPTION OF PLATES

and

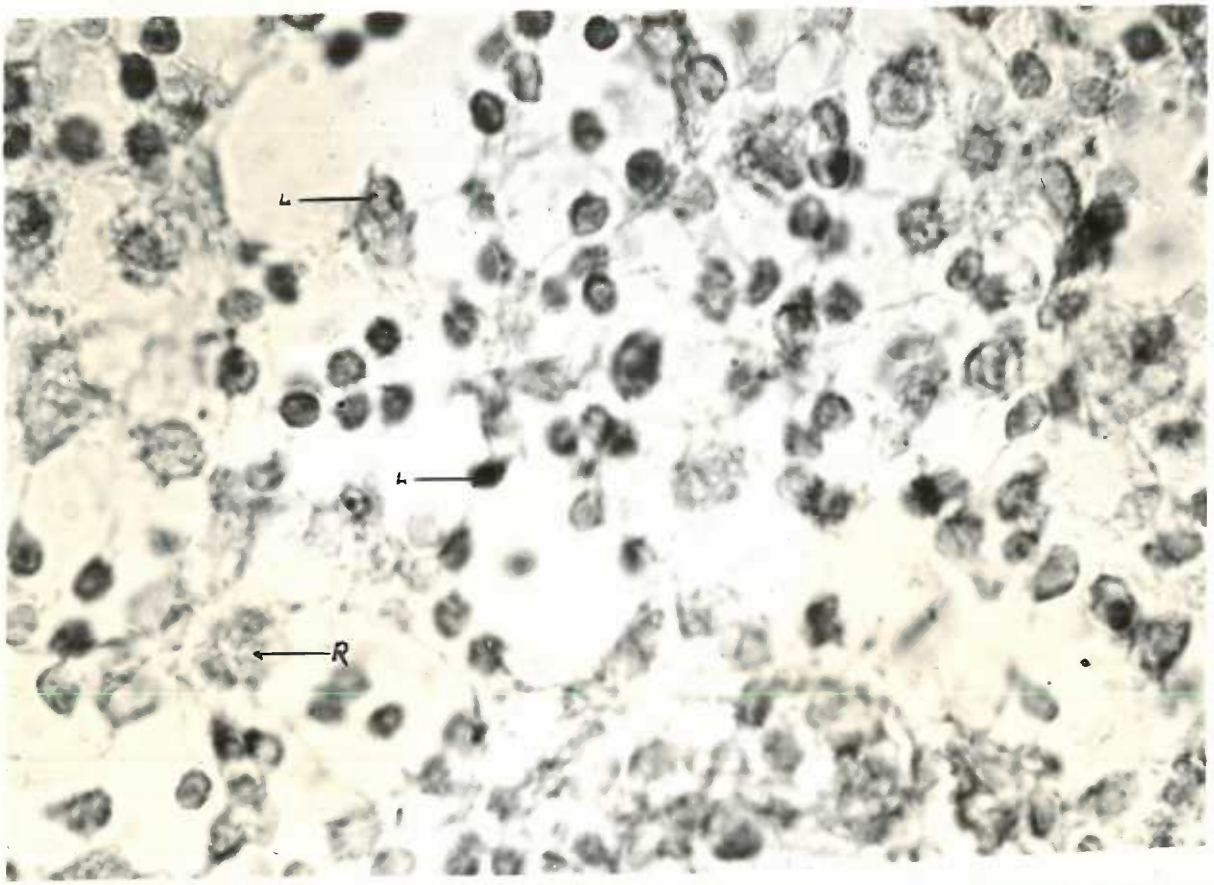
TABLE



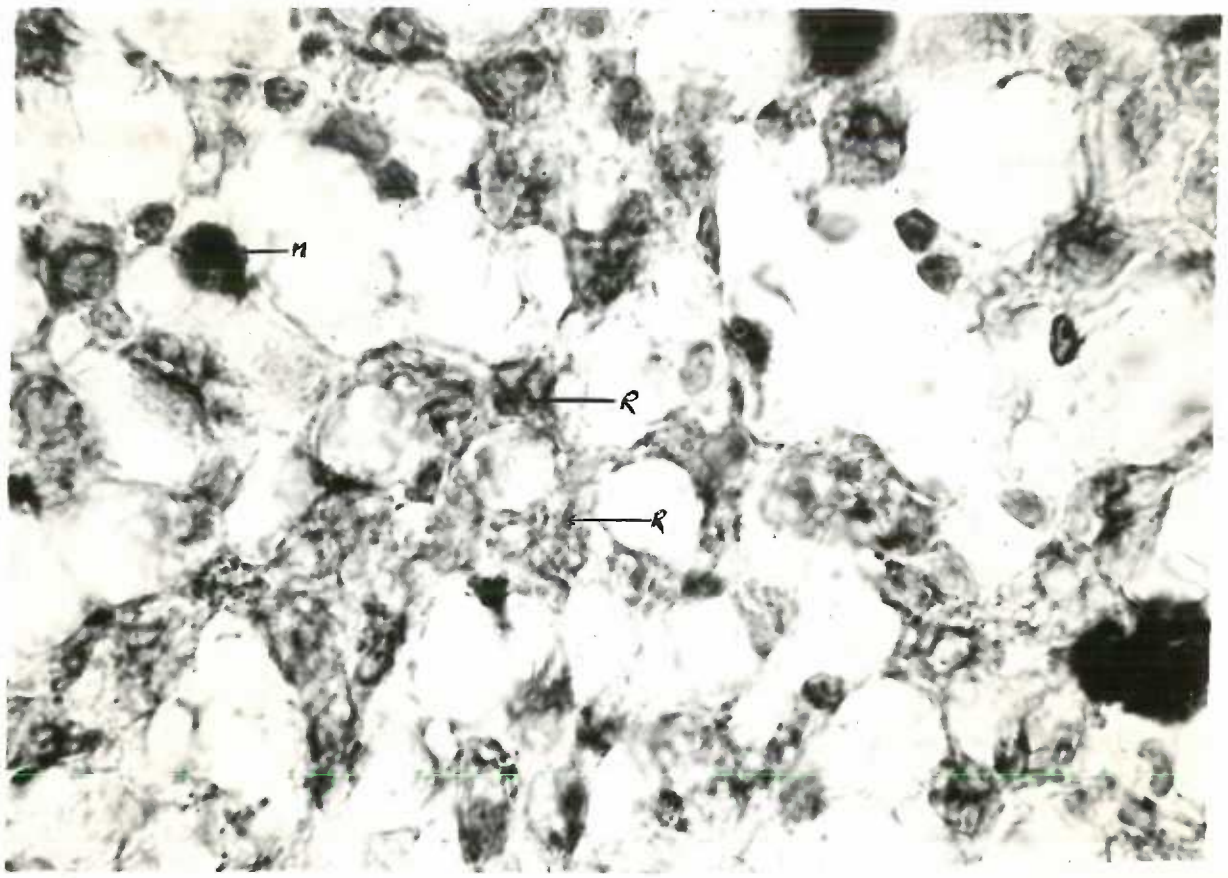
Lymph node of rat #51 experimental. Cells of the reticulum have stored a greater amount of trypan blue than those of the control animal. Compare with photograph of control on the following page. Large, darkly staining cells scattered throughout the tissue are mast cells. (safranin;7.5 micra; X 100) M, mast cell; R, cell of the reticulum.



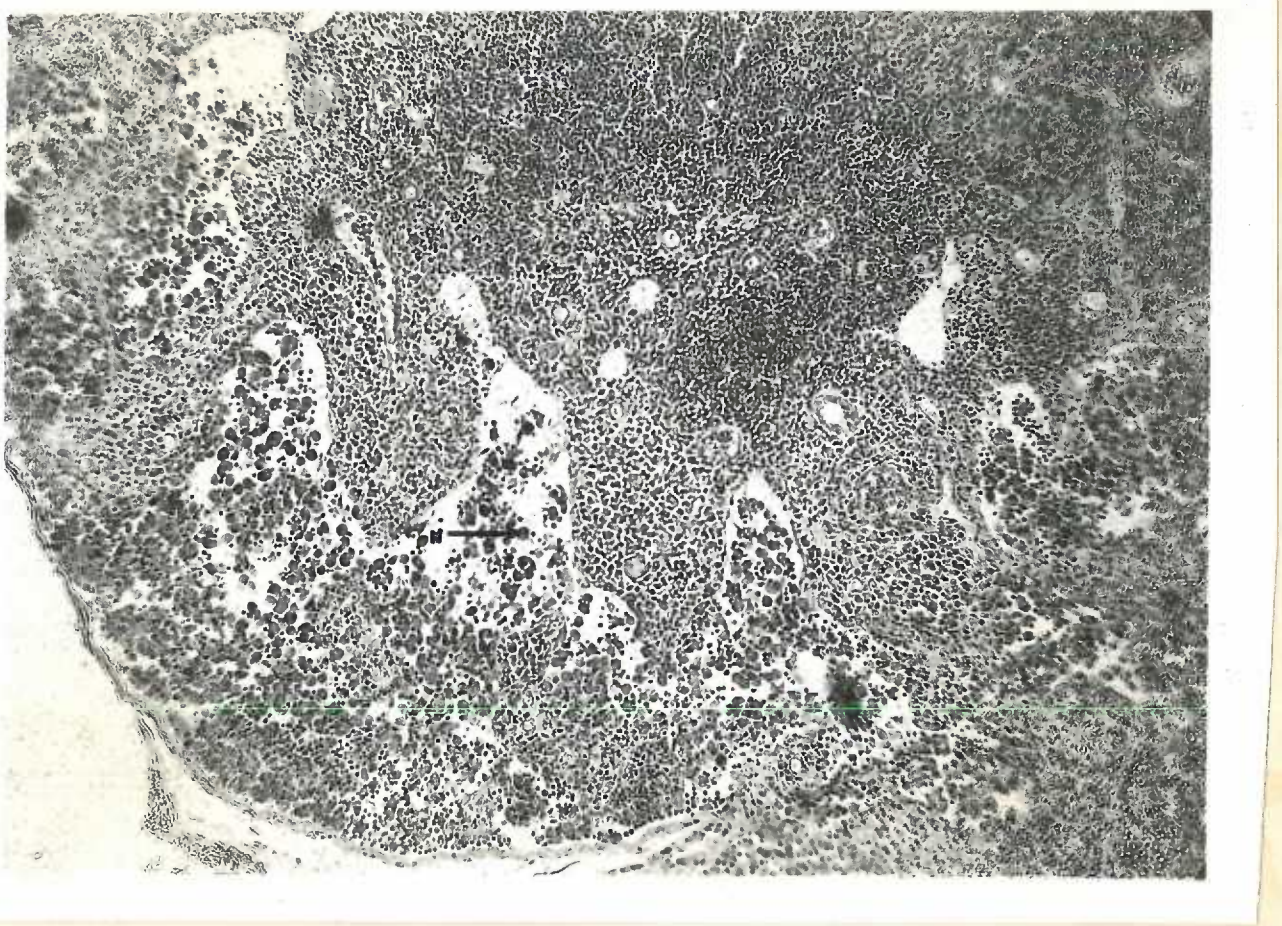
Lymph node of rat #31 control. Note cells of the reticulum in sinuses. Compare with photograph of the experimental animal. (safranin; 7.5 micra, X100) M, mast cell.



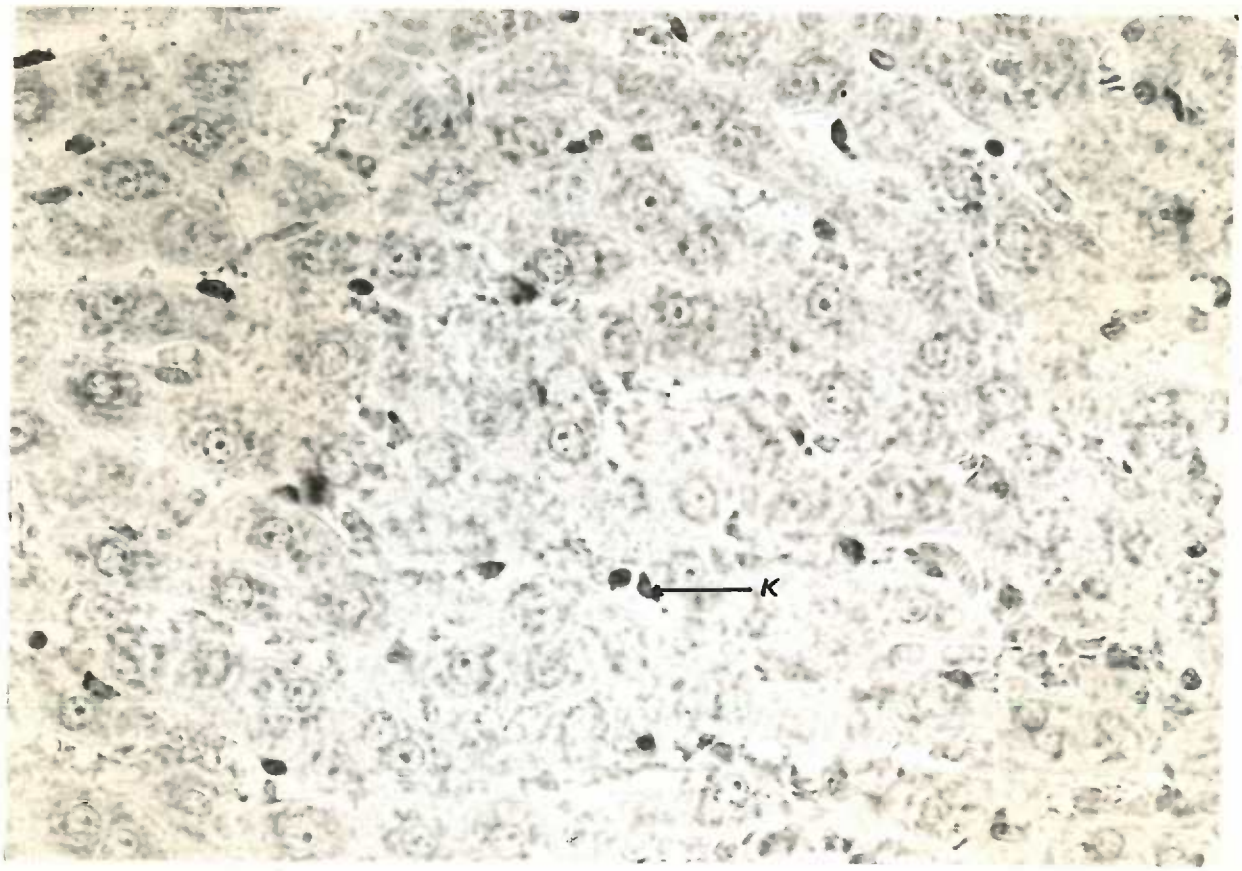
Lymph node of rat #31 control. Oil immersion view from the same section shown on page 52. Compare with photograph of the experimental lymph node on the following page. (safranin; 7.5 micra; X900)
L, lymphocyte; R, cell of the reticulum.



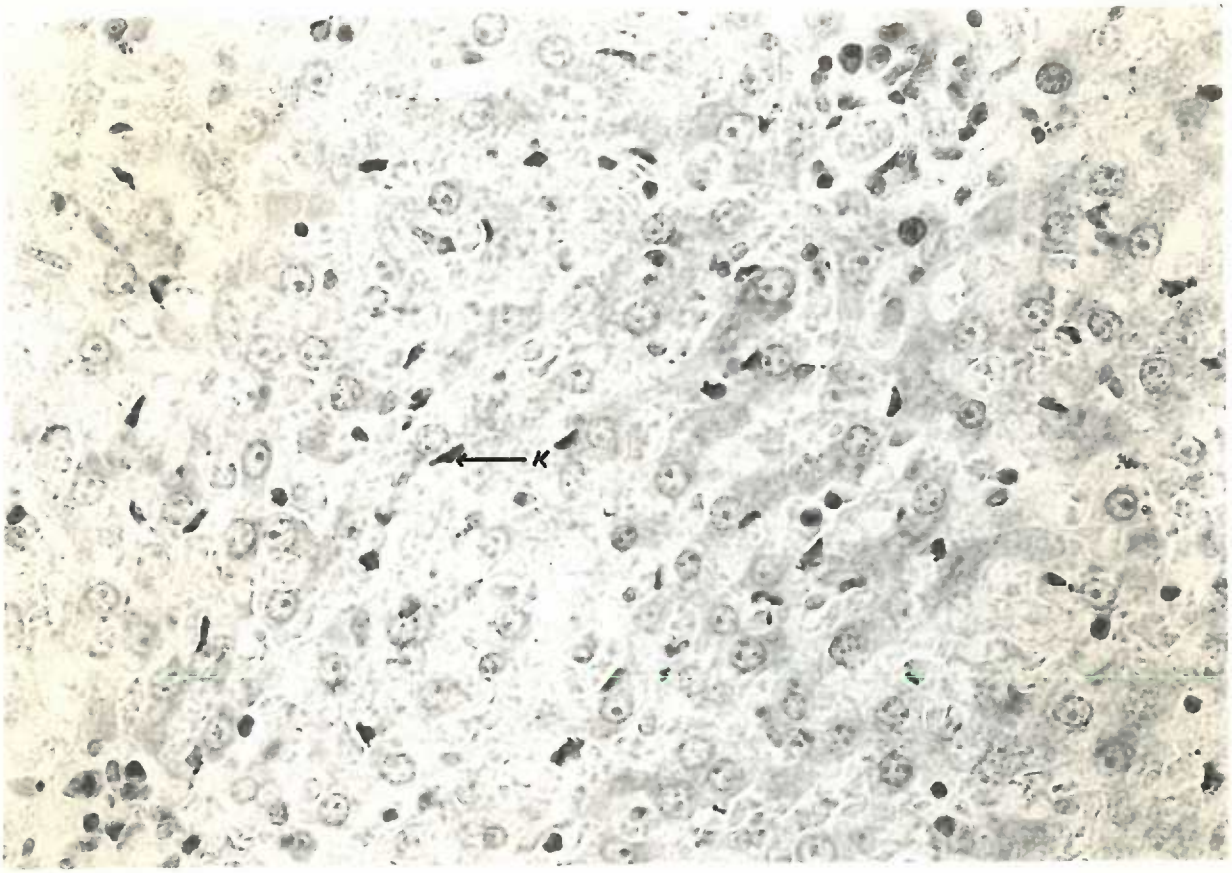
Lymph node of rat #31 experimental. Oil immersion view from the same section shown on page 41. Note fine granules of trypan blue stored by the cells of the reticulum which form a syncytium in the sinus. (unstained 7.5 micra; X900) M. mast cell; R. cell of the reticulum.



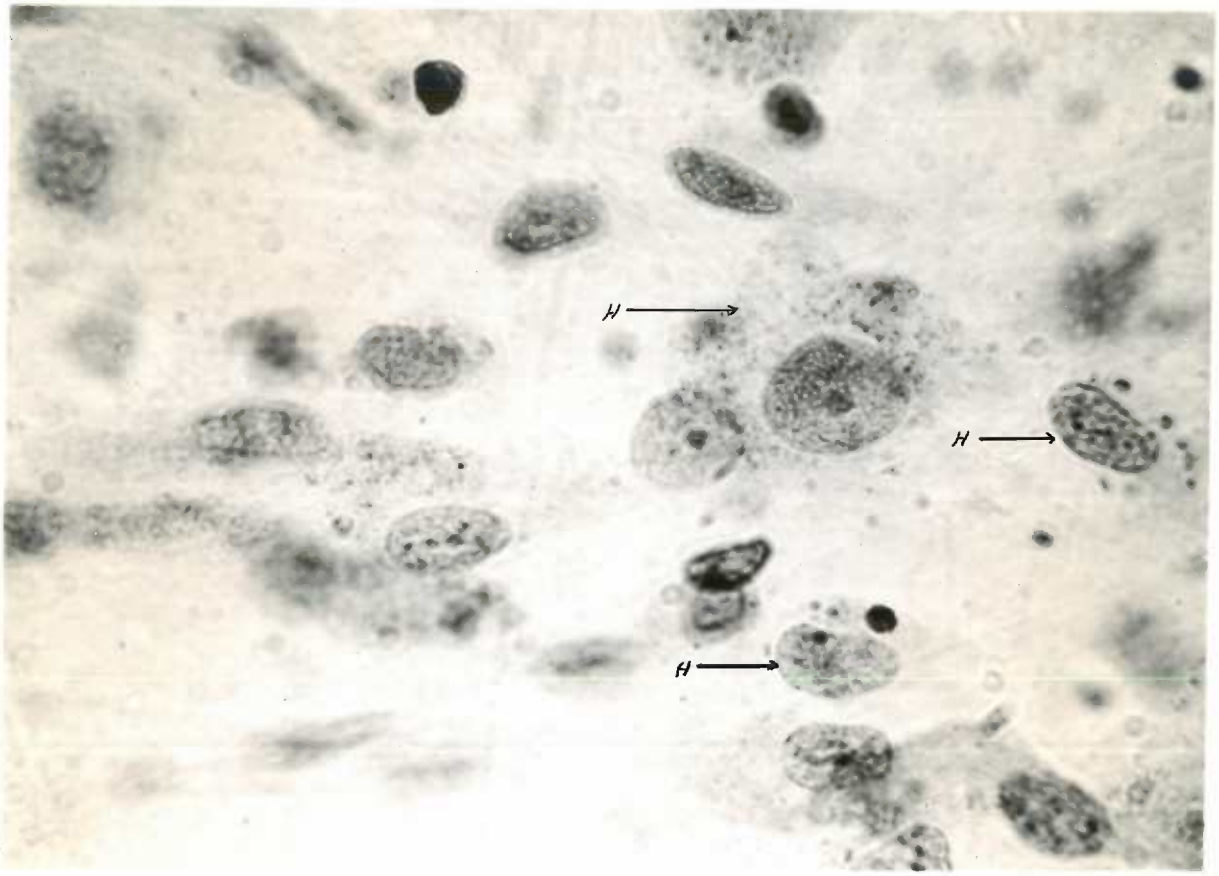
Rat #12 control. Lymph node demonstrating that the cells of the reticulum have rounded up into active, amoboid histiocytes under the stimulation of a heavy dosage of the vital dye. Compare with photographs of the lymph nodes of experiment #31. H, histiocyte. (safranin; 7.5 micra, X100)



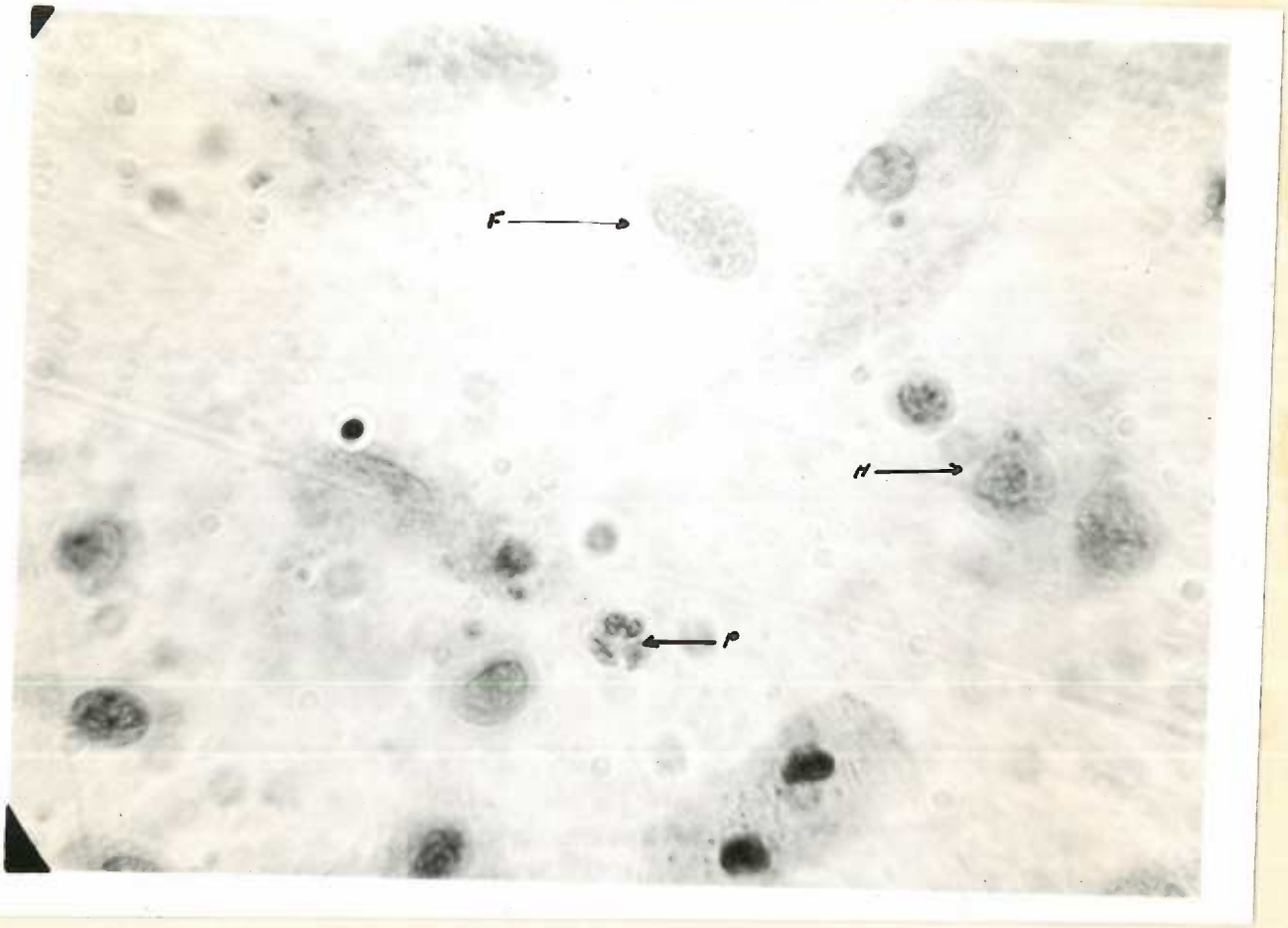
Rat #21 control. Liver showing Kupffer cells containing trypan blue granules. The small, dark cells in the sinuses of the liver are the Kupffer cells. (Safranin, 7.5 micra, X440) K, Kupffer cell.



Rat #21 experimental. Liver showing numerous Kupffer cells. In this experiment, the percentage of increase of the Kupffer cells over the control was 111%. K, Kupffer cell.
(safranin, 7.5 micra, X440)



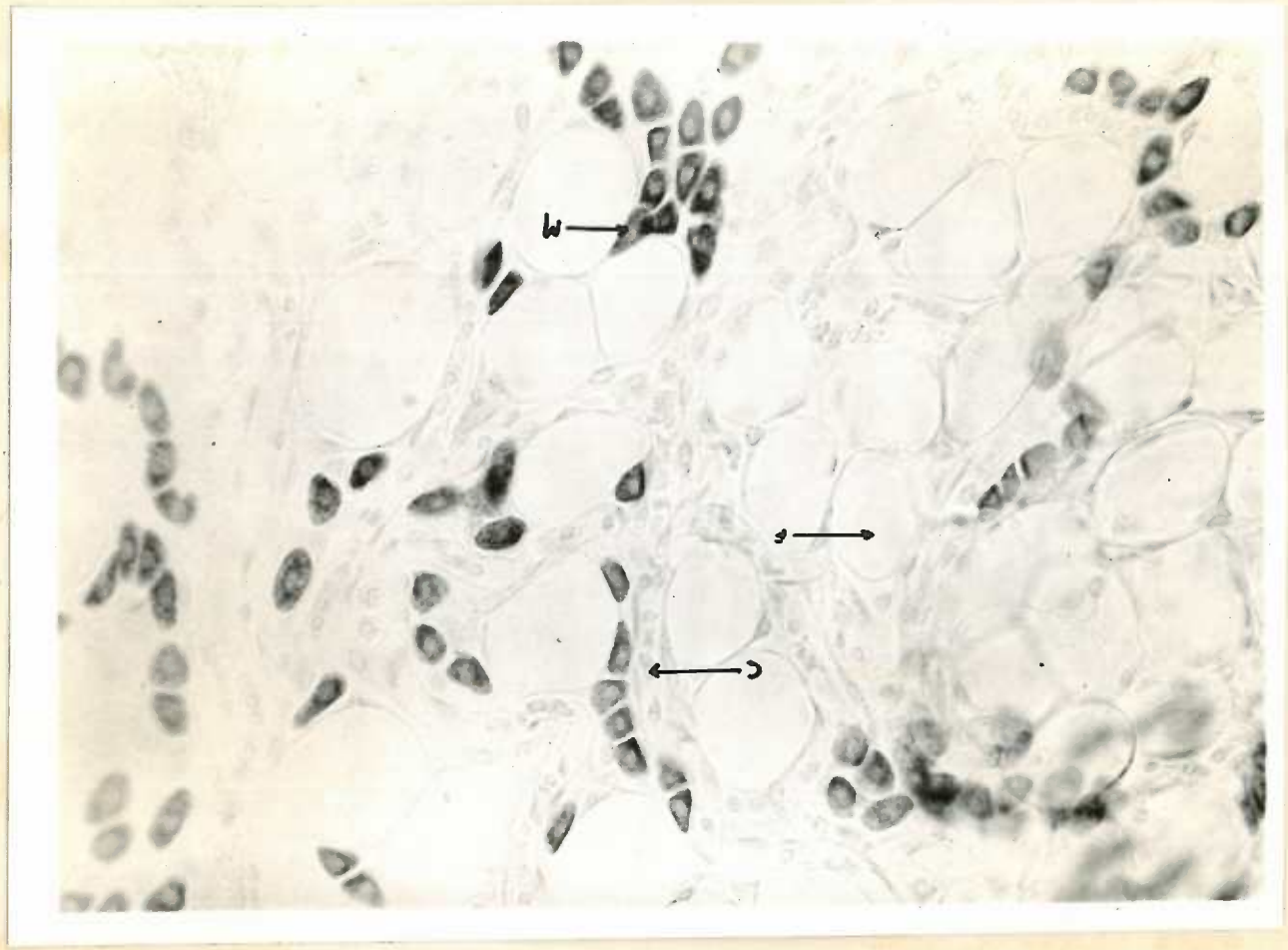
Rat #7 control. Stretch preparation of fascia. Note small size and number of trypan blue granules in cytoplasm of histiocytes in comparison with those of the experimental animal (page 68). (Iron-hematoxylin, X900) H, histiocyte.



Rat #7 control. A different field of the same stretch preparation of fascia as shown in the photograph on the preceding page.

F, fibroblast; H, histiocyte; P, polymorphonuclear leukocyte.

Rat #7 experimental. Stretch preparation of fascia. Note the numerous and large accumulations of trypan blue in the cytoplasm of the histiocytes. (iron-hematoxylin, X440) H, histiocyte.



Stretch preparation of omentum stained selectively for mast cells. Note grouping of mast cells around fat cells and capillaries. (X440)
C, capillary; F, fat cell; M, mast cell.

Average number of Kupffer cells per high power field. (Twenty fields were taken at random in sections of the same thickness from the livers of experimental and control animals.)

	Controls	Experimentals	
Number of experiment	Average no. of cells	Average no. of cells	Per cent increase
9	14	15	7%
18 (control for 13 and 16)	12		
13		16	33
16		22	83
19	14	24	71
20	10	17	70
21	17	36	111
30*	5	9	80
31	17	21	23
32	15	17	13
33	11	16	45

* Refer to description of experiment. Only cells which contained trypan blue granules alone were counted.

Typed by Katharine Mann