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EFFECTS OF RADIO-FREQUENCY ENERGY ON HUMAN GAMMA GLOBULIN

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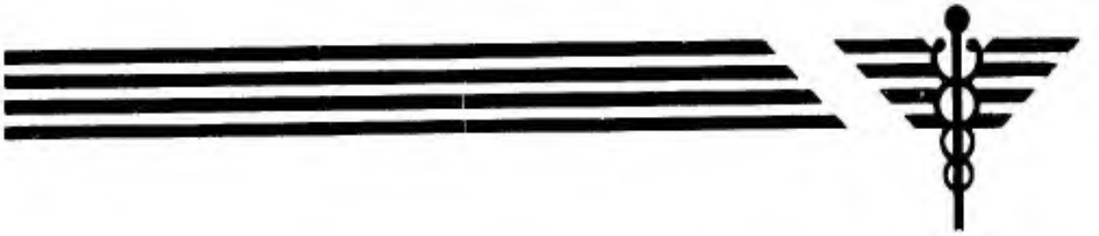
Biomedical Effects of Microwave Radiation
Task 01
Radiation and Thermal Burns
USAMRL Project No. 6X59-06-001



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ABSTRACT

EFFECTS OF RADIO-FREQUENCY ENERGY ON HUMAN GAMMA GLOBULIN

OBJECT

To determine whether or not specific energy absorption independent of heating can alter the characteristics of gamma globulin solutions exposed to radio-frequency energy.

RESULTS

Fourteen hundred (1400) individual exposures of 2.2 per cent gamma globulin solutions in normal saline with a phosphate buffer at a pH of 7.6 were made at frequencies between 10 and 200 megacycles per second. These exposures were carried out at a temperature at or below normal body temperature, at various frequencies and under varying conditions of voltage, pulse width, pulse repetition frequency, and temperature. It was found that changes in the electrophoretic pattern and in the antigenic reactivity of gamma globulin could be produced at certain frequencies. These changes could be produced at a suitable frequency at very low powers (1.6 milliwatts per square centimeter) provided the field strength was 5 volts per centimeter or greater and the frequency was suitable for the temperature during the exposure. Changes were produced at about 13 megacycles, 20 megacycles, and between 25 and 40 megacycles. The temperature dependence of frequency appeared to be about 2.4 per cent per degree centigrade.

CONCLUSIONS

One can alter the electrophoretic pattern and increase the antigenic reactivity of human gamma globulin by exposing it in vitro to radio-frequency energy of the proper frequency and field strength. The frequency depends on the temperature of the solution.

The temperature dependence of frequency appears to be of the order predicted by Debye's equation for relaxation times of polar particles in a viscous medium, 2.4 per cent per degree centigrade for water in the temperature range 30-40° centigrade.

Mass heating of the medium has no relationship to the changes. Neither does average power absorbed.

At 37.5°C in normal saline with phosphate buffer at a pH of 7.6, in the portion of the spectrum studied, the effective frequencies for human gamma globulin are near 13.1, 13.2, 13.3, 13.5, 13.6, and 14.4 megacycles. These may be the second harmonics in a series of harmonics which are also effective.

RECOMMENDATIONS

1. That the complete spectrum of response of the gamma globulins be thoroughly investigated.

2. That two other proteins obtainable in a pure (crystallized) form be studied by the same techniques in order to determine the role of molecular weight, size, and shape, the precise temperature dependence of frequency, the role of pH, ionic strength, presence of specific ions, and presence of other proteins.

3. That immunological investigations based upon the changes in antigenic reactivity demonstrated in this experiment be carried out on other antigens to determine whether or not antigenicity *in vivo* is also increased.

4. That changes in potency of the gamma globulins when used as antisera for various diseases be investigated.

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EFFECTS OF RADIO-FREQUENCY ENERGY ON HUMAN GAMMA GLOBULIN

I. INTRODUCTION

The absorption of electromagnetic energy in solutions of electrolytes, body fluids, and mammalian tissues has been well-documented throughout the r-f and microwave spectrum (1--3). Absorption of energy in these media, as measured by thermal rises, is a function of the complex dielectric constant and the conductivity. In the frequency range of 10 to 200 megacycles per second, in normal saline solutions with or without proteins in the usual concentrations found in the body, absorbed power is almost entirely a function of the conductivity and may be calculated on the basis of ohmic resistance and voltage for continuous wave signals, and ohmic resistance, voltage, and duty cycle for pulsed signals.

The interactions of electromagnetic energy with aqueous systems have been treated theoretically (4) on the basis of observed anomalous dispersions in dielectric constant and conductivity. Among these are dispersions due to orientation of polar particles in a viscous medium.

Microwave energy can alter the optical activity and cause precipitation in aqueous colloidal systems. Van Everdingen (5), in 1946, described gross alterations in optical activity of starch and glycogen solutions, and precipitation of starch solutions, by 3,000 megacycle radiation. These effects could be produced only at certain viscosities obtained by adjusting the concentration or by adding glycerol. Van Everdingen pointed out the importance of the viscosity term in Debye's equation for relaxation times of polar particles in a viscous medium, reasoning that at a certain combination of viscosity and frequency, maximal energy absorption would occur.

The paper electrophoretic pattern of human gamma globulin alters from a single peak to a double peak when exposed to high doses of X rays in vitro (unpublished data, Luzzio, USAMRL). These electrophoretic changes are accompanied by changes in antigenic reactivity as measured by titration against the serum of a rabbit immunized against X-irradiated human gamma globulin.

This article describes changes in the paper electrophoretic pattern and in the antigenic reactivity of human gamma globulin exposed to radio-frequency energy.

II. MATERIALS AND METHODS

Fourteen hundred individual exposures of gamma globulin[1] were made between 10 and 200 megacycles per second. Most exposures were between 10 and 40 megacycles. All were performed in 2.2 per cent-solution in normal saline or saline with a phosphate buffer at a pH of 7.6. The solution was placed in a small plastic chamber having two metallic sides, usually silver, which formed the electrodes to which the signal was applied. The electrode dimensions varied from long and narrow (5 by 1 cm) to square (1.5 by 1.5 cm). The chamber used most often had electrodes of 1.5 by 1.6 cm, spaced 3 mm apart. The energy source was a Hewlett-Packard Signal Generator controlled by a pulsing system in which pulses could be varied from a few microseconds (μ sec) in width up to 80 μ sec, and the repetition rate from 30 cycles per second (c.p.s.) up to 5,000 c.p.s. Most of the exposures were of 20-minutes duration, pulse widths of 10 or 60 μ sec and repetition rates of 500 to 2,000 c.p.s.

The pulsed energy from this source was amplified through a cascade of three low-power (Model 500A I.F.I.)[2] and two high-power (Model 400 I.F.I.) wide-band distributed amplifiers. (For the later low-power experiments only two of the low-power amplifiers were used.) From the final amplifier the signal passed through a power divider which enabled a reading of the power in each direction and hence a measurement of the voltage standing wave ratio (VSWR). The signal was then passed through a modified pi-network which could be tuned for minimum VSWR with a wide variety of loads. The VSWR in nearly all exposures was 1.02 or less. The output of this net was placed across the electrodes, one of which was grounded and cooled by a flow of constant temperature water. A shielded IN55A crystal shunted by a 39-Kilohm resistor was placed at the point of application of the signal to the ungrounded electrode. The half-wave rectified pulse passed through a transmission line to a Tektronix Model 545 Oscilloscope where the voltage, pulse width, and repetition frequency could thus be measured. The temperature of the solution was continuously recorded from a copper-constantan thermocouple held in place with a plastic jig and leading to a recording Brown potentiometer. The thermocouple was positioned halfway in depth and halfway between the electrodes. This position had been found to record the maximum temperature in the exposure chamber.

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[1] Gamma globulin used in this study was obtained through the courtesy of The American Red Cross.

[2] Instruments for Industry.

Power measurements were made in two ways. In the early experiments using air cooling, the thermal constant of the system was measured by first warming the solution with a continuous wave (cw) signal and then recording the temperature as it cooled. The equilibrium temperature difference between ambient air and the solution during an exposure and the heat capacity of the system then gave a measure of the average absorbed power.

With the later water-cooled system, the equilibrium temperature difference (ΔT) between power on and power off was found to be proportional to the square of the rectified voltage and to the pulse repetition frequency (p. r. f.).

Extrapolation of the p. r. f. -ΔT curves to zero p. r. f. at various voltages gave a measure of the ratio of pulse power to cw power between pulses and hence the ratio of the pulse voltage to cw voltage. This was found to be 30 to 1. Therefore, except at very low p. r. f's., the cw contribution to power was negligible.

A ΔT--power curve was obtained by measuring the change in temperature of the coolant water at a measured flow rate. Both types of measurement were checked against the calculated power using the following expression:

$$P = \frac{E_{eff}^2}{R} fw$$

where E_{eff} = 0.707 times the peak-to-peak voltage measured on the oscilloscope.

f = pulse repetition frequency in cycles per second.

w = pulse width in seconds.

R = resistance of load measured by a capacitance bridge at the operating frequency.

The capacitance bridge measurements showed, as expected, that the load was essentially a resistive one at these frequencies. The resistivity at 37.5° was found to be 50 ohm-cm.

Paper electrophoresis was carried out at room temperature with an LKB apparatus; 100 V at 4.0 ma being applied to the electrodes.

Schleicher and Schuell paper was used with a Veronal (diethylbarbituric acid)-sodium acetate buffer at pH 8.6, ionic strength of 0.125. Samples were applied to the paper strips by means of an applicator to which 12 microliters (μl) of protein solution had been delivered. Three controls and nine exposed samples were run in parallel. After sixteen hours the strips were removed and dried for thirty minutes in an oven set at 107 ± 2°C. The strips were stained for six hours in bromphenol blue dye at room temperature. This was followed by six washes in five per cent acetic acid. Fixing was for six minutes in acetic acid-sodium acetate. Subsequent to blotting and drying at 107°C, electrophoretic patterns were obtained by scanning in a Spinco Model R 110-115 V, 60 cycle Analytrol. Only those solutions which demonstrated a single peak by the above method were used for these experiments.

New Zealand albino rabbits were used for immune serum production. The animals received a first subcutaneous inoculation of 1 ml of human serum gamma globulin (22 mg protein/ml) followed by a second and third dose of 2 ml each of antigen administered intravenously on the third and fifth days. After seven days rest, the animals were bled by cardiac puncture under nembutal anesthesia. The blood was allowed to clot at 5°C and the serum was collected by centrifugation and decantation. Blood was collected every third week, and a booster inoculation of 45 mg of antigen administered one week after each bleeding. The collected serums were pooled and stored at -5°C in small quantities, then thawed for use as needed.

Precipitin titers were determined by the serial twofold dilution method. The technique consisted of layering 0.1 ml of the antigen diluted with saline on 0.1 ml of rabbit anti-human gamma globulin.

The first tube of each titration contained 22 mg of antigen per ml. The tubes were placed at room temperature for the first hour and 5°C for the second hour. Readings were taken at 15-minute intervals. The highest dilution which demonstrated a definite precipitin at the antigen-antibody interphase was recorded as the end point of the titration.

Thermal Controls: Gamma globulin solutions were immersed for 30 minutes in a constant temperature water bath at 33.5°, 37.0°, 42.0°, 48.0°, 52.6°, 57.2°, and 64.2° centigrade. A second set was immersed for 5, 10, 15, and 20 minutes in the water bath at 55.0°, 60.0°, 62.0°, 63.0°, and 64.2° centigrade.

III. RESULTS

Thermal Controls: Most samples showed no visible changes. All samples at 64.2°C and the 15- and 20-minute samples at 63.0° developed precipitates. Paper electrophoresis showed that samples with no precipitate were similar to the controls. The samples with precipitates showed higher and sharper peaks. No double peaks were seen.

The first exposures were at 10-megacycles increments between 10 and 200 megacycles for 30 minutes each. At this time water was not used for temperature control. With only air cooling the temperature rises during exposure were less than 5°C. In Figure 1 the final temperatures attained are seen to be 30°C or less, well below normal body temperature. On paper electrophoresis most of the samples showed no change. However, at 30, 60, 140, 180, and 200 megacycles, gross changes in the form of a distinct double peak were seen. The average powers in this series, based on the temperature rises, which were 5°C or less, and the thermal time constant of the exposure chamber, were less than 300 milliwatts (60 mw/cm^2 average power density). There was no correlation between temperature rise and effect.

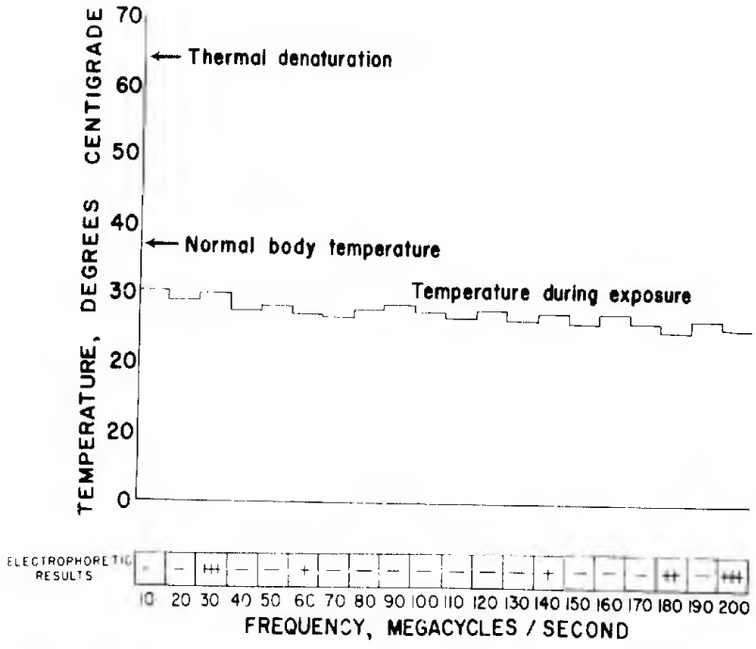

Fig. 1. Changes in gamma globulin solutions exposed to r-f energy at about 60 milliwatts per square centimeter for 30 minutes each. Negative signs indicate no change in electrophoretic pattern. Plus signs show degree of electrophoretic changes on a one to four-plus subjective scale.

The region near 30 megacycles was then explored in 1-megacycle increments. These exposures were all at 13 volts rectified, (26 V peak-to-peak) 1196 cycles per second, and 10 microseconds pulse width. The field strength was 87 volts per cm and the exposure time 15 minutes. Since, by measurement on the capacitance bridge, the load was found to be 3 ohms, the calculated power was:

$$\frac{(26 \times 0.707)^2}{3} (1196)\ (10^{-5}) = 1.35 \text{ watts}$$

$$\frac{1.35}{5} = 0.27 \text{ watt/cm}^2, \text{ the electrode dimensions being } 1 \times 5 \text{ cm}$$

Figure 2 shows the results with the corresponding time-temperature curves. It was apparent that the electrophoretic changes were not profound and that the band width under these conditions was 13 and 17 per cent of the frequency (4-5 Mc in 30). A repetition gave positive results at 29.0, 31.0, and 34.0 megacycles, the changes being greater.

A limited range was then explored at 5 per cent frequency increments. In all these exposures the temperature varied between 30 and 40° centigrade. The grounded electrode was cooled by flowing water.

Each of the exposures within an individual run was at the same voltage, pulse width, pulse repetition rate, power, and duration; only the frequency varied. Exposures were randomized within each run. Some typical exposure parameters for these runs are shown in Table 1.

Table 1. Exposure conditions for experiments at 5 per cent frequency increments and random temperatures between 30-40° centigrade

Watts/cm^2 Average Power Density	Pulse Repetition Frequency Cycles/sec	Pulse Width μsec	Field Strength Peak-to-Peak Volts/cm	Exposure Time Minutes
3.4	2000	10	240	20
4	3300	10	200	20
3.3	1780	70	93	20
2	1667	10	200	20
4.8	1000	10	400	20

It was obvious from the results that conditions were not being controlled well enough between exposures and that 5 per cent frequency

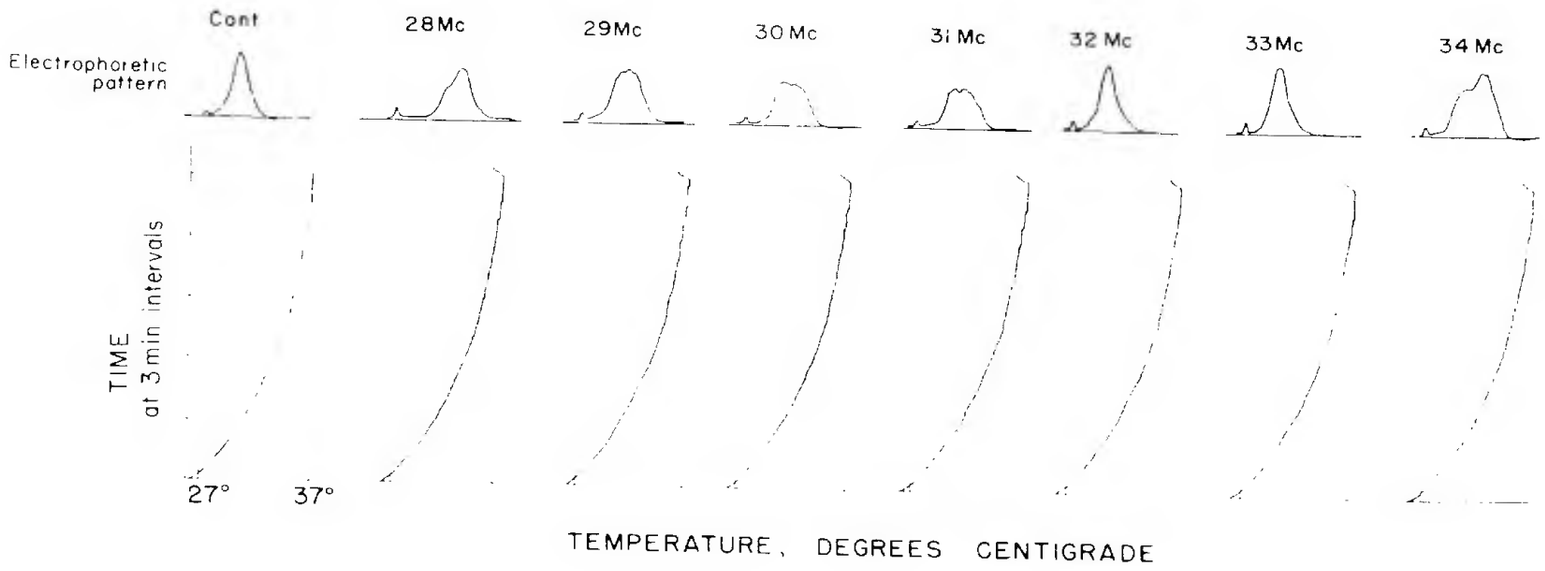

Fig. 2. Tracings of electrophoretic patterns and temperature curves of gamma globulin solutions exposed for 15 minutes each at 0.27 watt per square centimeter. Cont. indicates control which was placed in the same chamber as exposed samples and carried through the same time-temperature history by warming in an incubator. Each dotted line shows 37° for the temperature curve beginning at its left.

increments were too large since sometimes no effect was obtained. After six sweeps at 5 per cent increment between 10 and 41 megacycles it was, however, apparent that the successful exposures were grouped in some sort of pattern, and that this pattern shifted with temperature. The experiments of Van Everdingen with starch and glycogen furnished a guide to the degree of frequency shift with temperature.

Debye's equation for relaxation times of polar particles in viscous media is $\tau = \frac{3V\eta}{kT}$ (see footnote 3)

If one takes the empirical equation for visocity of a liquid; $\ln \eta = \frac{a}{T} + b$ (footnote 3) and combines it with Debye's equation then $f = CTe^{-(\frac{a}{T} + b)}$ (footnote 3)

On differentiation, the change in frequency with temperature in a water solution can be calculated. This turns out to be 2.42 per cent per degree centigrade. An earlier experiment in which both electrodes had been cooled with diesel fuel, at 22°C, thus keeping the temperature constant with a very low gradient, furnished a basis for analysis of the data. In this experiment effects had been obtained at 10.0, 15.0, 20,0, and 25.0 megacycles which seemed to indicate that harmonics of 5 megacycles were involved.

Assuming a fundamental frequency of 5 megacycles to be effective at 22°C and the effective frequency to change 2.4 per cent per degree centigrade, then the frequency ranges of the effective harmonics can be calculated at other temperatures. Figure 3 shows these ranges between 30° and 40° centigrade. If one then assumes a certain probability of obtaining a detectable effect in an effective frequency range under the exposure conditions for this series, say 0.25, (which was the experience figure) and further assumes that the points of overlap in the harmonics will increase the chances of success ($P_2 = 1-q^2$), then the theoretical probabilities of success appear as shown at the bottom of the

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[3] η = viscosity, k = Boltzmann's constant, V = volume of spherical particle, f = frequency, c, a, b, are constants, τ = relaxation time, and T = absolute temperature.

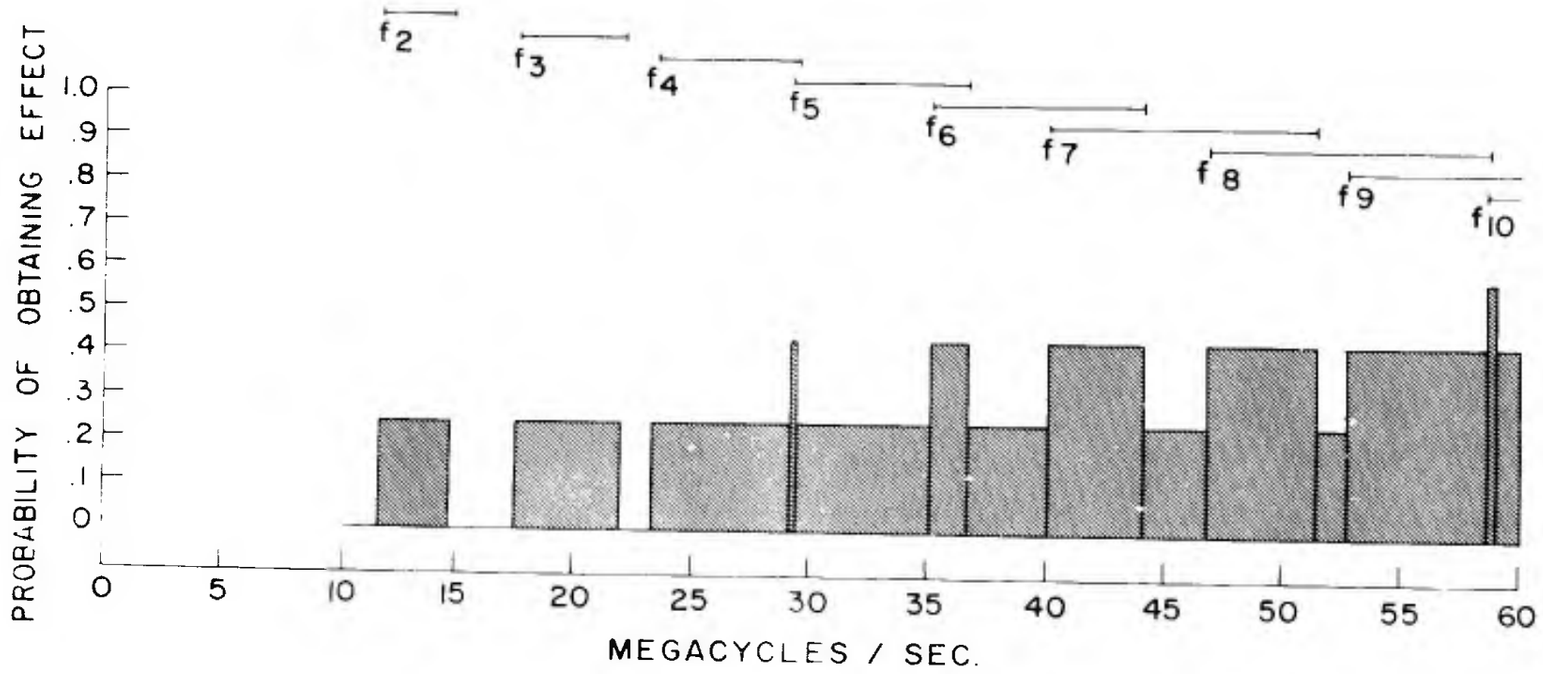

Fig. 3. Hypothetical probabilities of obtaining detectable effects of 5% frequency increments and random temperatures of 30 to 40°C. Based on calculated ranges of effective harmonics in this temperature range.

figure. Taking these probabilities and applying them to the actual frequencies of exposure (Figure 4) one obtains a theoretical pattern of successes marked "Hypothetical" in this figure. The summary of the data is shown at the bottom. A rather striking agreement is seen between theoretical and actual results. These considerations would lead one to surmise that harmonics of around six megacycles were involved in this process, the exact frequency depending on temperature.

Since the equipment did not go below 10 megacycles the f_2 harmonics calculated to be somewhere between 12.4 and 13.5 megacycles were used as a basis for the next exposures in that range at 100 Kc increments, once at a relatively high power and high temperature gradient in the cell and again at about one half the temperature gradient. The recorded temperature was maintained at 35.1°C throughout each exposure. These runs (Figure 5, page 12) showed a narrowing of the band width without a decrease in the effect when the temperature gradient was decreased. Seven repetitions of these exposures at the lower power gave positive results which showed rather poor reproducibility. However, a correlation was found between effective frequency and the ambient temperature and humidity during exposure; the frequency being lower on cool dry days and higher on warm wet days (Figure 6, page 12). Subsequent exposures were made in a humidified incubator and the reproducibility at once improved, the shifts in effective frequencies being less than 1 per cent between runs.

From the data at 35.1°C, a calculation, based on 2.4 per cent shift in frequency per degree centigrade, was made to predict results at 37.5°C. The predicted effective frequencies were 13.1, 13.2, 13.3, 13.9, 14.3, and 14.4 megacycles. The next run at 37.5°C produced changes at 13.10, 13.20, 13.30, and 14.30 megacycles.

The frequency increment was then reduced to 20 kilocycles and with careful crystal calibration of the generator between each exposure, the range between 13.00 and 13.34 megacycles was explored. Double peaks were obtained at 13.12 and 13.32 megacycles. Six replications showed a similar pattern with 20-40 Kc shifts in the effective frequencies about the points 13.10, 13.20, and 13.30 megacycles.

The actual electrophoretic patterns for the first run are shown in Figure 7, page 13. It is immediately apparent why this effect is so exasperatingly elusive. At 20 kilocycles on either side of this gross change, there is no hint of anything

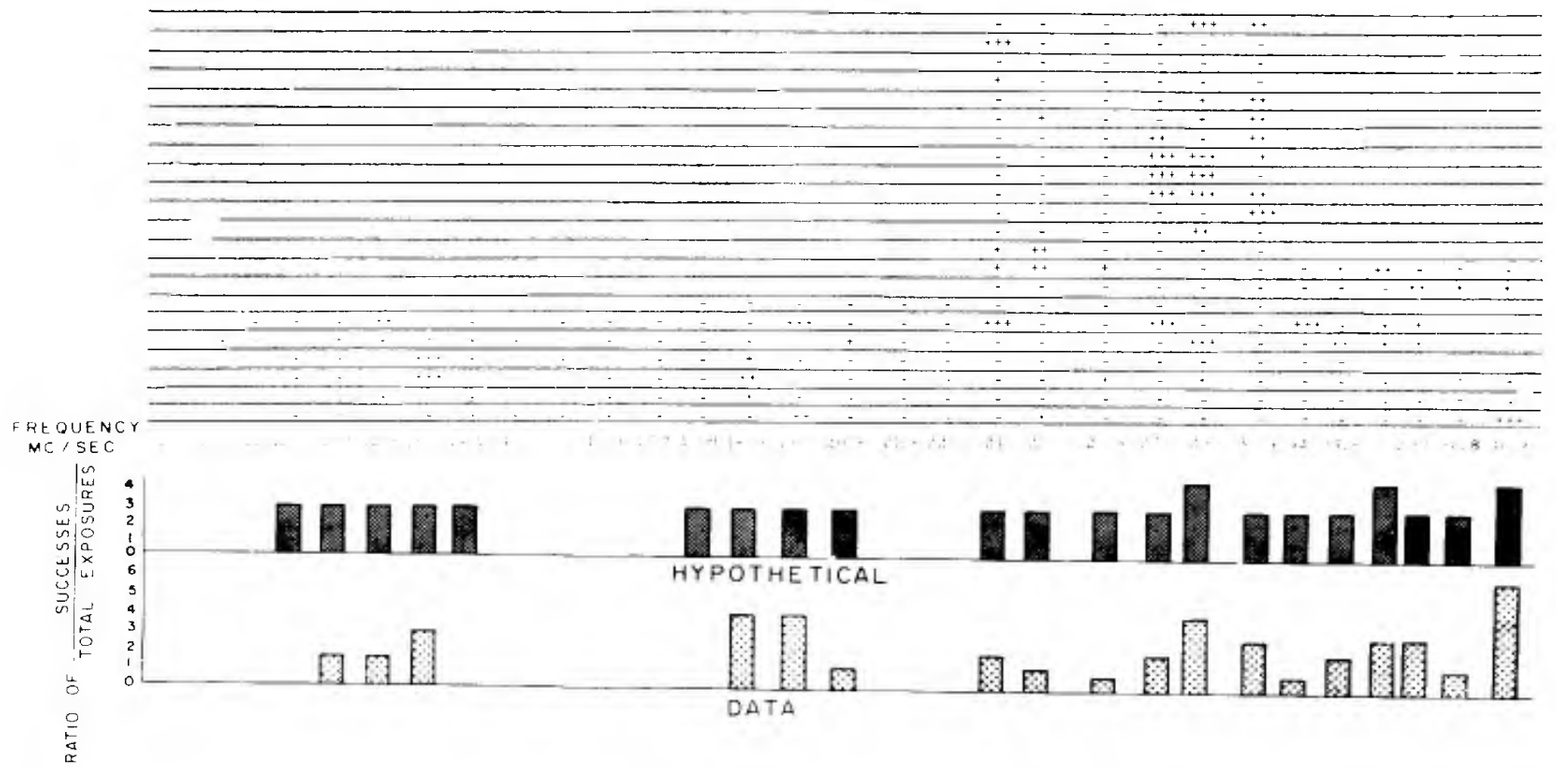

Fig. 4. Summary of results of electrophoresis on gamma globulin samples exposed between 3(and 40° centigrade. Comparison of actual successes with theoretical pattern of success.

Temp. Gradient °C	Pulse Repetition Frequency	FREQUENCY Mc/sec											
		12.4	12.5	12.6	12.7	12.8	12.9	13.0	13.1	13.2	13.3	13.4	13.5
6-7°C	1040	+++	+++	+++	++++	+++	-	-	-	-	-	-	-
.3-3.5°C	525	-	++++	++++	+	-	-	-	-	-	-	+	-

Fig. 5. Effect of temperature gradient in exposure chamber. Plus signs indicate degree of electrophoretic changes on a one to four-plus subjective scale. The recorded temperature was 35.1° C, pulse width 60 μ sec, field strength 133 Volts/cm, and the duration 20 minutes in each case.

Relative Humidity	FREQUENCY Mc/sec											
	12.4	12.5	12.6	12.7	12.8	12.9	13.0	13.1	13.2	13.3	13.4	13.5
≤ 54	+	-	-	-	-	-	-	-	-	-	-	-
55 - 60	+++	+++	+++	-	-	-	-	+	-	-	-	-
61 - 65	-	++++	++++	+	+++	+++	-	++	-	-	-	-
				++++				-	-	-	-	-
66 - 70	-	-	-	0	-	0	-	-	-	-	+	-
≥ 71	-	-	-	-	-	++++	-	+	++	-	++	++++ ++++

0 = No data

RELATIONSHIP OF FREQUENCY TO RELATIVE HUMIDITY

Fig. 6. Relationship of frequency to ambient relative humidity and temperature. Electrophoretic changes in gamma globulin exposed in an uncontrolled temperature and humidity environment at constant recorded temperature of 35.1° C. The days with low relative humidity were cool and the wet days were warm.

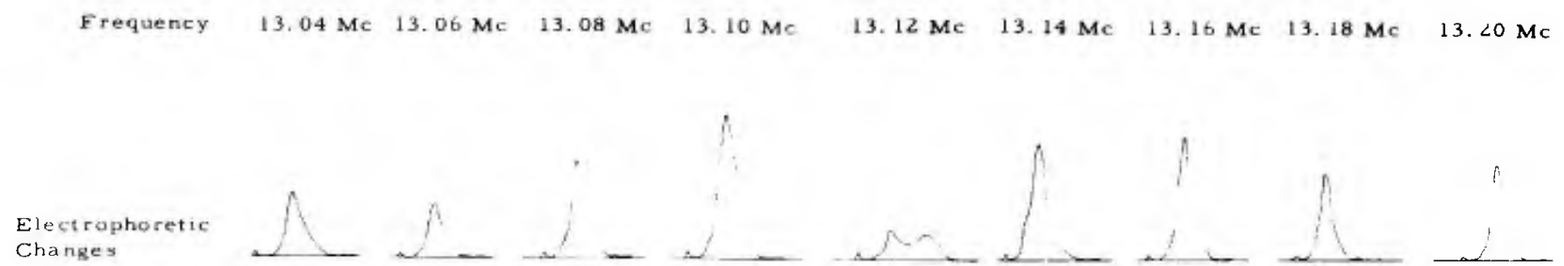

Fig. 7. Narrow banding effect of constant temperature exposure with ambient temperature and humidity controlled. Tracings of electrophoretic patterns of gamma globulin exposed at 37.5°C.

occurring except perhaps the high peaks. This is a frequency increment of about 0.15 per cent, corresponding to a temperature shift of 0.06°C.

In order further to characterize the changes and to assign some biological meaning to them, the sensitive and highly specific techniques of immunology were next applied. Exposed samples of human serum gamma globulin and non-exposed controls were titrated against the serum of rabbits which had been immunized against non-exposed human gamma globulin. Twenty exposed and five unexposed samples were titrated against non-immune rabbit serum, to determine the presence or absence of precipitins for human serum gamma globulin. Results were negative.

Against the immune serum, surprisingly, significant increases in titer occurred in samples exposed at 13.10, 13.20, 13.30, 13.50, 14.30, and 14.40 megacycles at 37.5°C whereas the others at every 100 kilocycles, between 12.8 and 14.5 megacycles, showed no significant differences from the controls. Several replications showed that these high titers occurred on each side of the frequencies at which the electrophoretic double peak appeared. For example, in the sample showing a double peak in Figure 7 the titer was not significantly different from the control while at 60 Kc below and 120 Kc above, the titers were sixteen times that of the controls. Figure 8 shows the results of two runs at 100 Kc increments, at 37.5°C, between 12.8 and 14.5 megacycles.

Taking a titer of four times the control as significant, it appeared that there were three regions in this part of the spectrum centering at 13.2, 13.5 or 13.6, and 14.4 megacycles where the changes occurred. These agreed with the regions of electrophoretic change at this temperature, though, as already mentioned, the individual samples showing double peaks did not have a high titer, but rather were adjacent in frequency to the high titer samples.

Since the spectrum between 12.8 and 14.5 megacycles showed several regions of effective frequency at 37.5°C, with regions of electrophoretic change adjacent to those of high titer, it seemed likely that more power than necessary was being used for these exposures. Accordingly, a limited range, between 13.04 and 13.39 megacycles was explored in 10-kilocycle increments with the results shown in Figure 9, page 16. Then the upper portion of this range was chosen for an experiment at reduced power and field strength. The results of these exposures, with the exposure parameters, are shown in Figure 10, page 17. In the exposures at 13.4 milliwatts/cm^2 and below, there

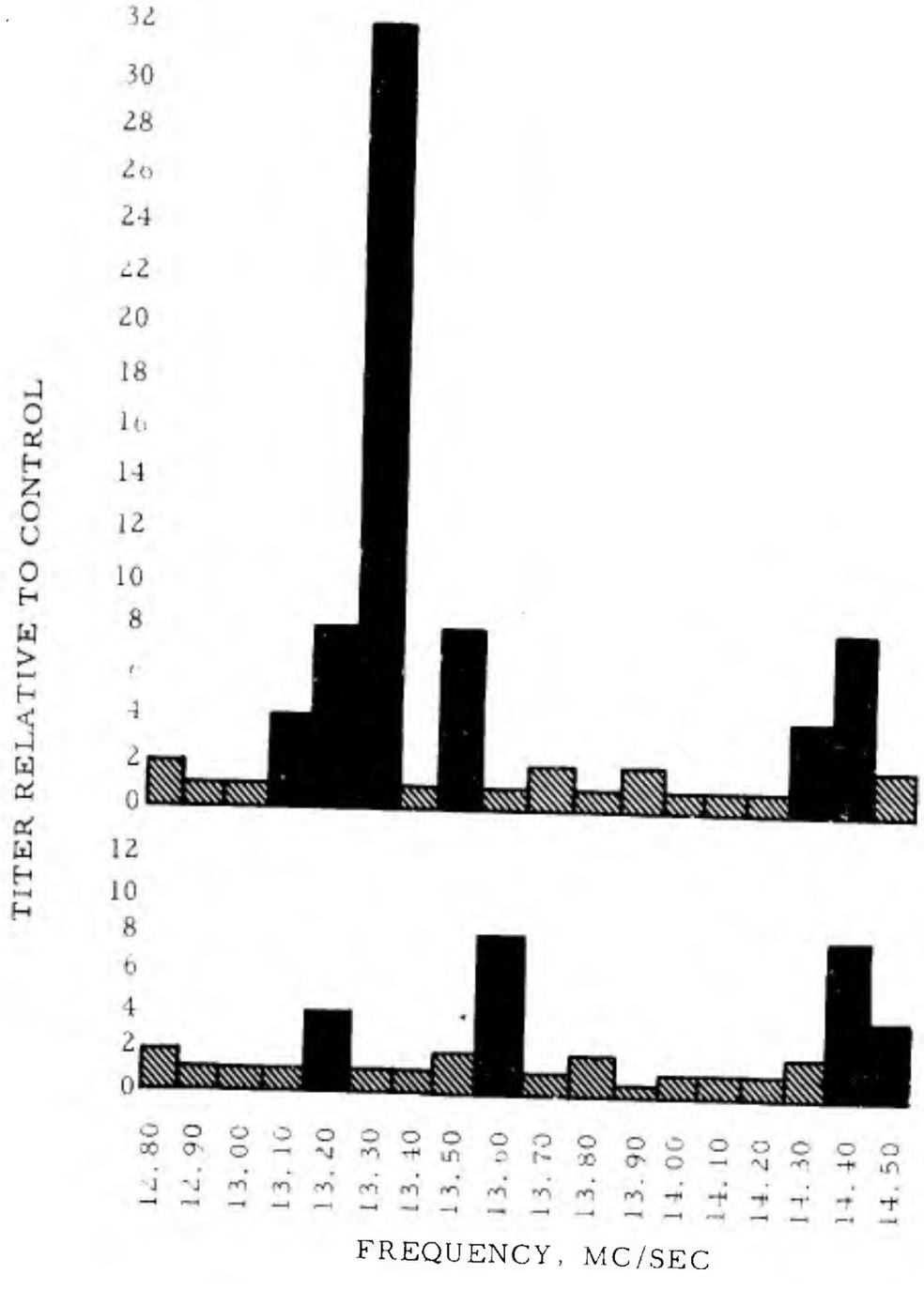

Fig. 8. Relative titers of gamma globulin exposed to r-f energy in 100 Kc. increments between 12.8 and 14.5 megacycles at 37.5°C. Titers are shown in their ratio to the control titer when tested against serum of a rabbit immunized against human gamma globulin.

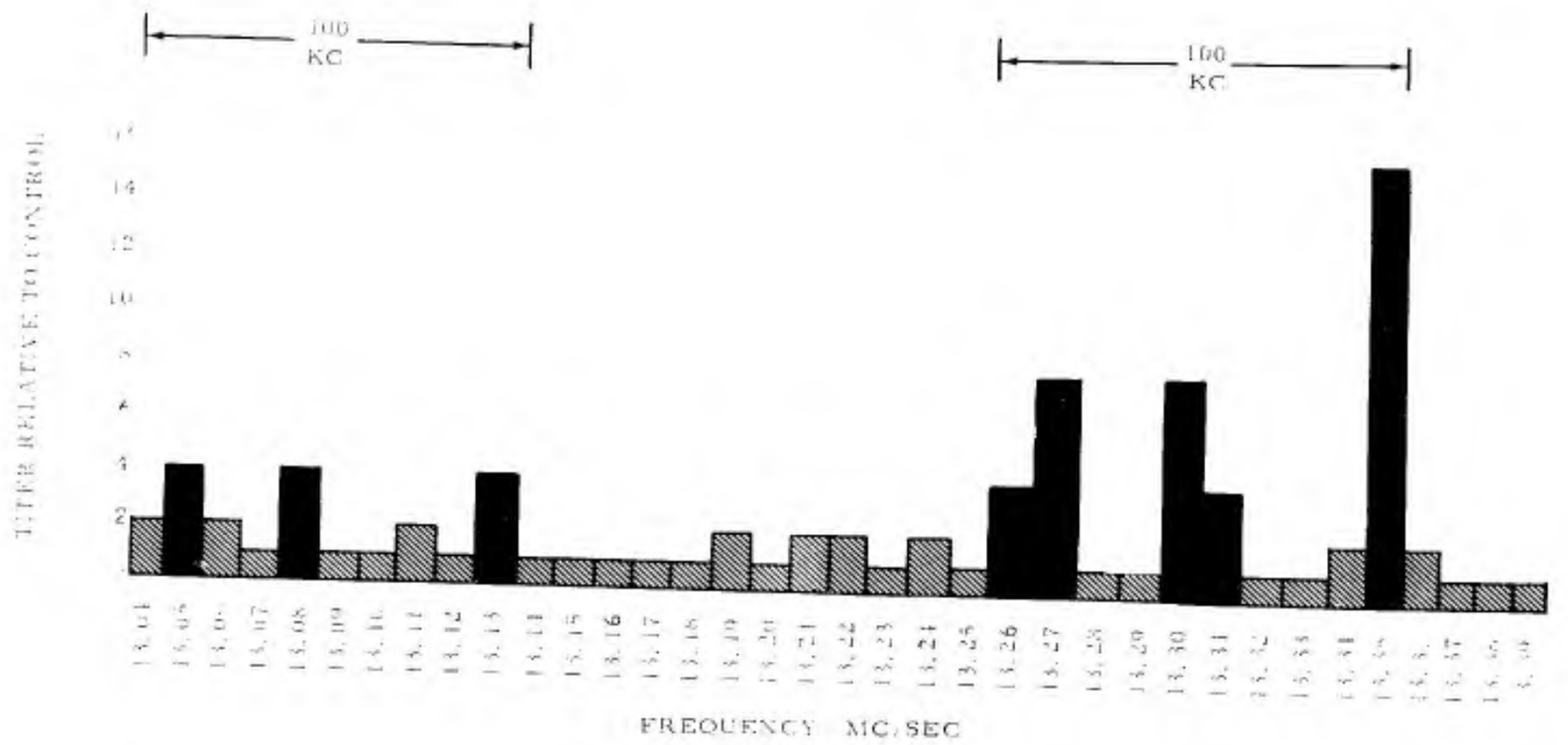

Fig. 9. Relative titers of gamma globulin exposed to r-f energy in 10 Kc. increments at 37.5°C. Note that this is a very narrow portion of the spectrum shown in Fig. 8.

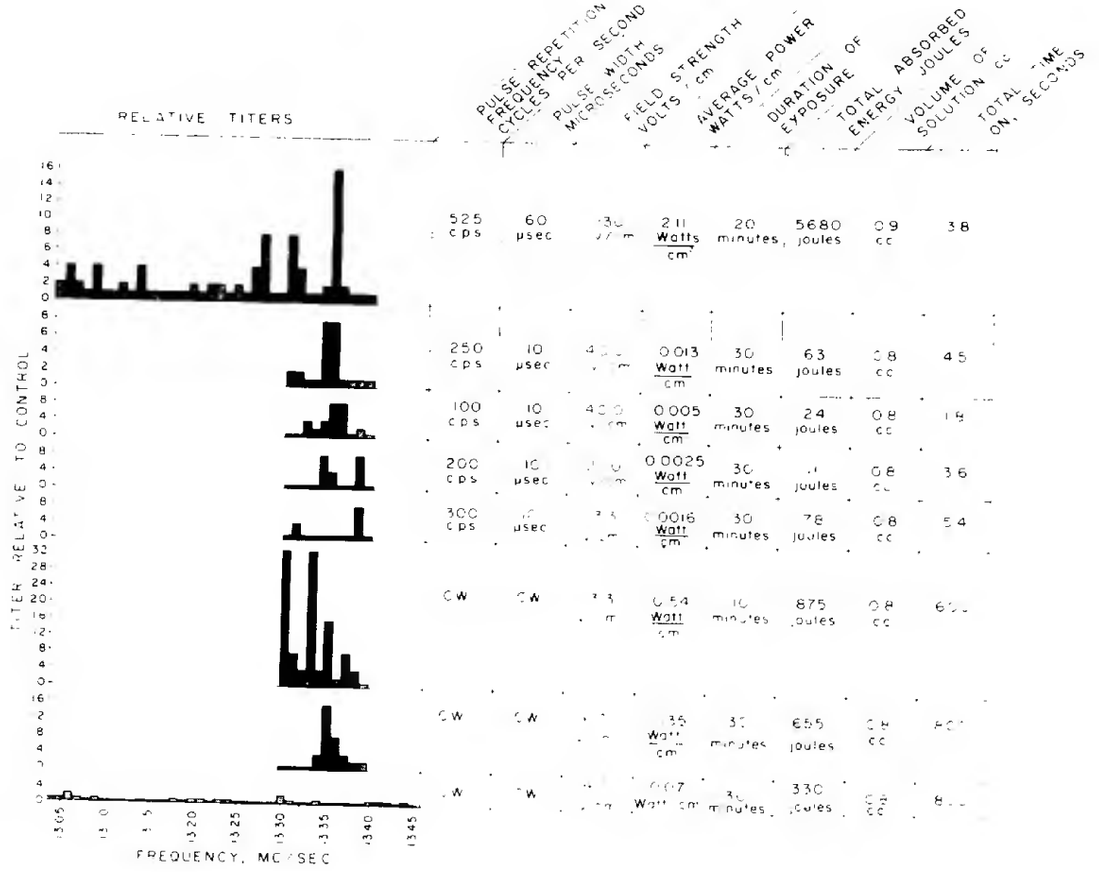

Fig. 10. Effects of reduced power and field strength. Relative titers of gamma globulin exposed in a narrow frequency range at various powers and field strengths. Note that positive effects are obtained at powers as low as 1.6 milliwatts per square centimeter. At 44 times that power (70 milliwatts per square centimeter) no effect is obtained when the field strength is lowered to 4.7 volts per centimeter.

was no detectable temperature rise when the power was turned on. All of the temperature control was due to constant temperature water circulating in the grounded electrode. It should be noted that effects were produced even at 1.6 milliwatts/cm^2. However, if the field strength was reduced to 4.7 volts/cm, even 70 milliwatts/cm^2 was not effective.

IV. DISCUSSION

Changes in the electrophoretic pattern and antigenic reactivity of human gamma globulin took place when this protein was exposed to r-f energy. These changes were produced only at certain combinations of frequency and temperature. They occurred at temperatures well below that required for thermal denaturation.

If exposures were made at very high power and field strength (960 watts per square centimeter peak instantaneous power density, 4.8 watts per square centimeter average power density, and 400 volts/cm field strength) at an unsuitable frequency and temperature combination, no changes were produced at all.

If, however, the frequency was suitable for the temperature of the exposed solution, gross changes in the electrophoretic pattern and the antigenic reactivity occurred even at very low power and field strength (0.22 watt per square centimeter peak instantaneous power density, 1.6 milliwatts per square centimeter average power density and 13.3 volts/cm field strength).

If the field strength was reduced to 4.7 volts/cm, even at a suitable frequency and temperature, no changes were produced, even though the power was increased 42 times over that which was effective at 13.3 volts/cm.

The production of changes at temperatures well below normal human body temperature, the complete lack of correlation of effect with temperature rise or with average power and its dependence on frequency and field strength, rule out mass heating of the medium as a factor in producing these changes.

One can regard the samples exposed at high power and field strength but the "wrong" frequencies as the true controls for these experiments since they were exposed to the same kind of energy under the same conditions as the samples which were altered. These unchanged samples serve to rule out non-specific effects which might be invoked such as electrode surface effects. Also, such effects are

ruled out by the fact that in chambers of different electrode surface areas, the changes occurred at the same frequencies.

From the nature and degree of the shift in effective frequency with temperature, it is likely that the macroscopic viscosity and the absolute temperature both determine the frequency response. The interaction taking place is probably one involving the whole molecule, perhaps a rotation in the alternating field. The shape and size of the molecules as well as the temperature and viscosity of the medium may be important in determining the frequencies at which these effects are obtained.

From the changes in antigenic reactivity one might theorize that this energy produces some degree of unfolding of the protein helix, laying bare more specific combining sites. With increased exposure to an effective r-f frequency, the molecules may become so grossly altered as to lose their specificity. This notion is borne out by the fact that at high power the gross changes in electrophoretic pattern occurred near, but not at, the frequencies at which maximum titers occurred.

The pattern of successful exposures at random temperatures between 30 and 40°C is highly suggestive of a harmonic series whose fundamentals are between 5.97 and 7.42 megacycles at these temperatures.

V. CONCLUSIONS

One can alter the electrophoretic pattern and increase the antigenic reactivity of human gamma globulin by exposing it in vitro to r-f energy of the proper frequency and field strength. The frequency depends on the temperature of the solution.

The temperature dependence of frequency appears to be of the order predicted by Debye's equation for relaxation times of polar particles in a viscous medium, 2.4 per cent per degree centigrade for water in the temperature range 30-40°C.

Mass heating of the medium has no relationship to the changes. Neither does average power absorbed.

At 37.5°C in normal saline with phosphate buffer at a pH of 7.6, in the portion of the spectrum studied, the effective frequencies for human gamma globulin are near 13.1, 13.2, 13.3, 13.5, 13.6, and 14.4

megacycles. These may be the second harmonics in a series of harmonics which are also effective.

VI. REFERENCES

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