# SOME EFFECTS OF ULTRAHIGH FREQUENCY INDUCED ELECTRICAL CURRENTS ON CERTAIN MICRO-ORGANISMS

A thesis

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# LIST OF ILLUSTRATIONS

# I

## ACKNOWLEDGMENTS

The author wishes to take this opportunity of thanking the following for inspiration and for technical and scientific advice and aide as is in each case appropriate: Professor W. A. Murray of the Electrical Engineering Department; Professor P. H. McGauhey and Mr. H. F. Eich of the Sanitary Engineering Department; Professor F. S. Orcutt and Mr. J. R. Leonards of the Biology Department at Virginia Polytechnic Institute.

We also thank Mr. J. R. Winston of the United States Department of Agriculture for supplying the six crates of oranges used for experimental purposes.

## II

## INTRODUCTION

High frequency induced electrical energy has been used by medical science for several years to produce heat internally in the human body. The uses of such a process are many and varied. They range all the way from producing artificial fever in the entire body to heating one certain afflicted spot such as a rheumatic joint.

Experiments have been performed in the past to determine the effects of high frequency induced energy on certain micro-organisms. To the best of the authors knowledge, Fabian and Graham of Michigan State College[1] have made the most thorough and conclusive investigations.

The experiment as performed by the author was inspired by the results obtained at Virginia Polytechnic Institute recently by the treatment of several bottles of fresh

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1. Fabian and Graham 1933
   "Influence of High Frequency Displacement Currents on Bacteria"
   Journal of Infectious Diseases 53:76-88

apple cider.[2] Unfortunately insufficient data was recorded to make it possible to duplicate the experiment using other substances, including culture, instead of cider. Although the results obtained by the author were negative, they seem to be of sufficient importance to record.

Upon first consideration, the theory behind the experiment seems to be sound. Let us assume a micro-organism extremely exaggerated as in figures 1 and 2. If an alternating voltage of say 32 million cycles per second is applied to the plates, a high frequency electric field will be set up between them. Suppose plate "A" is negative and plate "B" is positive at any given time as shown in Fig. 1. Then all of the electrons and negative ions will migrate toward plate "B" and all of the positive ions toward the "A" plate. If the frequency is $32 \times 10^6$ cycles per second, then the condition as shown in Figure 2 will exist $\frac{1}{64 \times 10^6}$ second after the condition shown in figure 1.

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2. Vaughn, Revercomb, Murray, and Whittemore 1940

"Drying Ceramic Products by use of Induced High Frequency Electrical Energy"

Bulletin of the Virginia Polytechnic Institute Engineering Experiment Station. No. 42

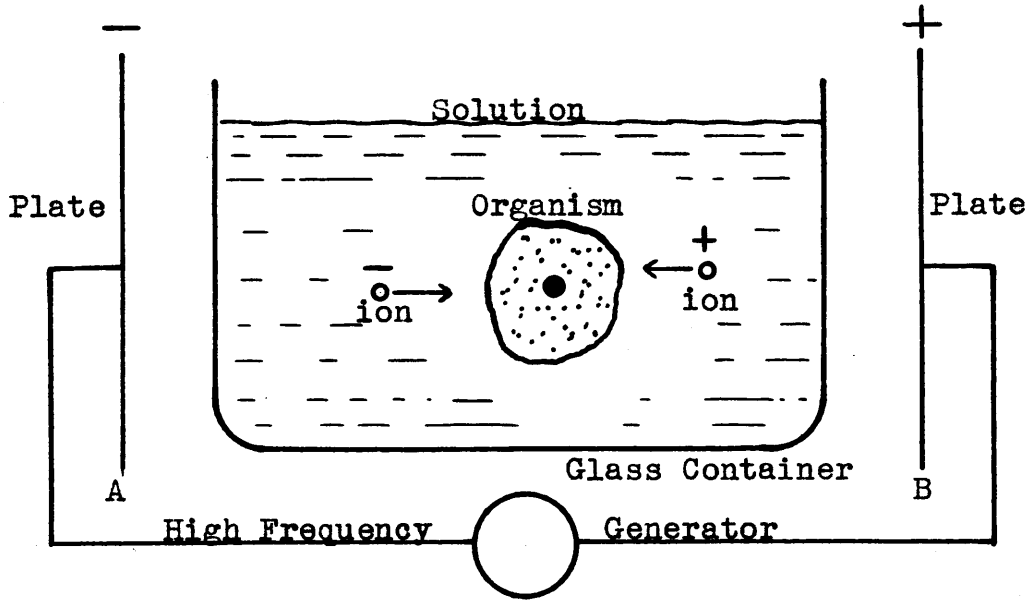


Fig. 1

Migration of ions at any instant when "A" is negative and "B" is positive.

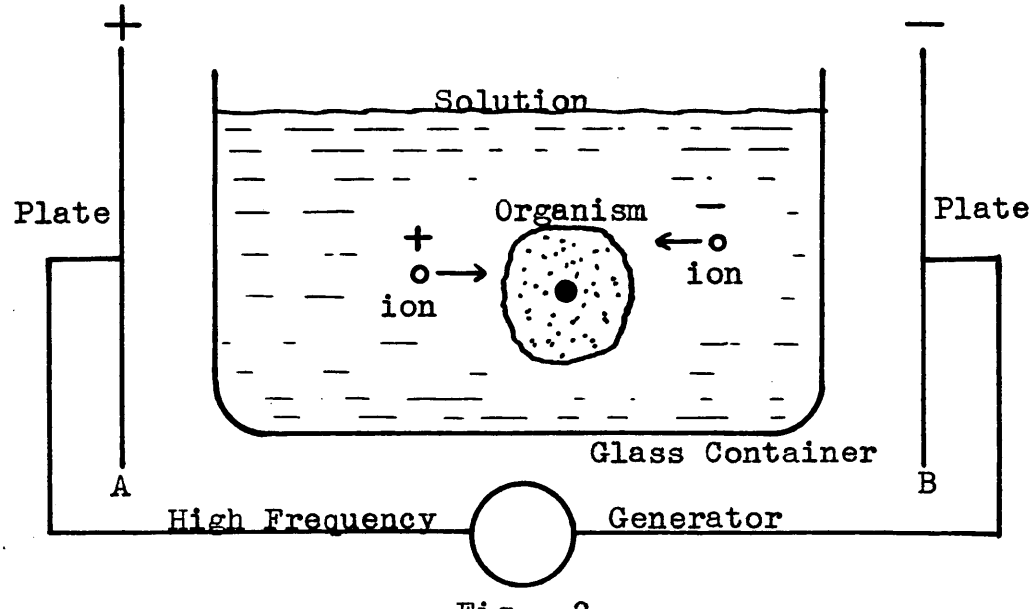


Fig. 2

Condition existing $\frac{1}{2f}$ seconds after the condition shown in Fig. 1.

This migration of ions constitutes an electric current. Apparently this bombarding of the organism with positive and negative ions should produce some effect other than heat.

This work was undertaken to test this process with the view of possibly destroying certain organisms, especially those causing fermentation.

## III

## REVIEW OF LITERATURE

When a high frequency voltage is applied to the plates of a condenser, there is considerable ionic displacement in the dielectric material between the plates. This ionic and electronic movement necessarily generates heat. It, in effect, constitutes a flow of electric current; thus the name "induced currents". We know that an electric current generates heat.

This induced current may easily be seen to be proportional to the voltage across the plates and to the degree of ionization or the conductivity of the dielectric. Thus we see that a highly ionized solution will take more power and heat faster than a material ionized to a lesser degree. Obviously the heating effect will not be the same for organic and inorganic salts or for colloidal and noncolloidal materials.

If organisms are to be affected by a high frequency field, then the effect will be different for different solutions in which the organisms are suspended. That is, if the organisms are suspended in a saline solution, different results may be obtained if the same

organisms are used in a different solution, every other factor remaining constant.

There seems to be a wide field for experimentation in this work, because there are so many variables which might easily determine the results. For example, if an organism is to be killed, there is certainly a definite frequency, a definite solution, a minimum time of exposure, and, perhaps, a minimum power input to the solution for maximum killing effect.

A uniform electric field would seem to be desirable. Fabian and Graham[3] used cylindrical electrodes as shown in figure 3. This would give a concentrated field near the center electrode. They obtained approximately 90 per cent sterile solution after eight hours of treatment at 10 megacycles. In their experiment arrangements were made to keep the solution cool by means of circulating water.

Detailed information concerning this test may be found in the reference given in footnote 1, page 2.

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3. See footnote 1, given on page 2.

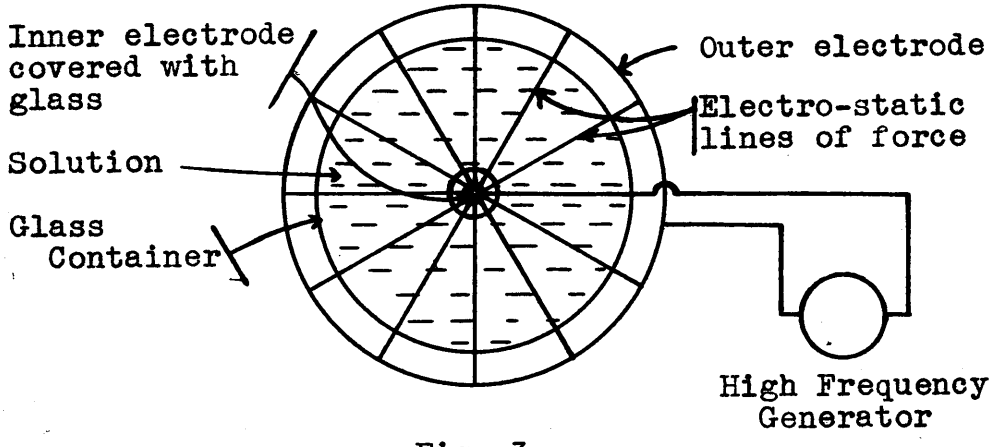


Fig. 3

Figure showing schematically the arrangement of the electrodes and the load in the tests run by Graham and Fabian. Note the concentration of the lines of force near the center electrode.

For very good references on electric fields and dielectrics, the reader is refered to entries 1 and 3 in the bibliography of this work.

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# IV

## APPARATUS

The oscillator used to produce the high frequency voltages for this test is essentially the same as the one used by Mr. Rivercomb and Mr. Vaughn in 1938-1939, and by Mr. Hitt and Mr. Wright in 1939-1940, for their respective experiments with ceramic products.

The oscillator circuit is of the push-pull power oscillator type and is of Westinghouse origin, having been developed by Mr. H. J. Dailey of that company.

Figure 4 shows a complete diagram with the following notations:

P - Copper plates between which the load is placed.

$L_2$- Plate Tank--5 turns of $\frac{1}{4}$ in. copper tubing, $3\frac{1}{2}$ in. in diameter, $3\frac{1}{2}$ in. long.

$L_3$- Choke coil--38 turns of No. 14 copper wire, $\frac{1}{2}$ in. in diameter, 2 3/4 in.long.

$L_4$- Choke coil--38 turns of No. 14 copper wire, $\frac{1}{2}$ in. in diameter, 4 in. long.

$L_5$- Grid Tank--4 turns of 1/8 in. copper tubing, $1\frac{1}{2}$ in. in diameter, 4 in. long.

$T_1$- Filament Transformer--110 to 10 volts, 10 amperes.

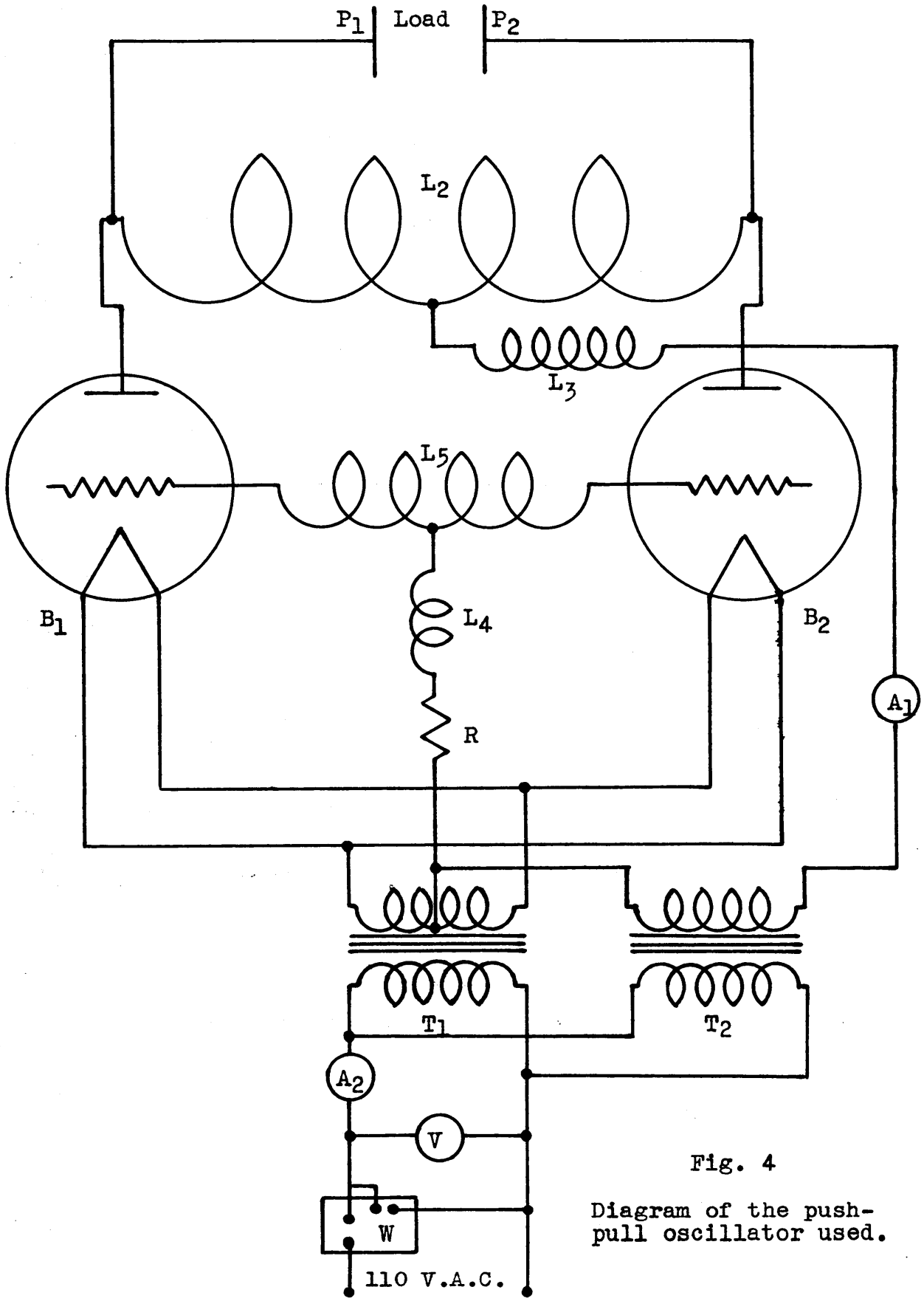


Fig. 4

Diagram of the push-pull oscillator used.

$T_2$- Plate Transformer--110 to 2300 volts, 5 kva.

$A_1$- D. C. Milliameter--500 ma.

$A_2$- A. C. Ammeter--25 amps.

V - A. C. Voltmeter--150 volts.

W - A. C. Wattmeter--.75 to 1.5 kw.

R - 5000 ohm resistor.

B - Three Electrode Vacuum Tubes rated as follows:

| | |
|---|---|
| Plate Potential (RMS) (A.C.)-- | 2500 volts |
| Plate Current (D.C.)---------- | 0.200 amps. |
| Plate Input---------- | 550 Watts |
| Plate Dissipation---------- | 150 Watts |
| Grid Potential (D.C.)--------- | -500 volts |
| Grid Current (D.C.)---------- | 0.060 amps. |
| Grid Current (R.F.)---------- | 8.0 amps. |
| Grid Potential (R.F.)--------- | 750 volts |

Plate Output (approximately)

| Frequency | Watts |
|---|---|
| 6 megacycles | 400 |
| 15 megacycles | 350 |
| 20 megacycles | 325 |

This circuit has the very convenient advantage of requiring alternating voltage only. The main disadvantage is that the frequency is very hard to vary.

# V

## INVESTIGATIONS

### A. The Oranges

One of the chief causes of decay in Citrus fruits is the stem-end rot. The following quotation from a bulletin issued by the United States Department of Agriculture gives a clear insight into the nature of the rot:

> Stem-end rot of Florida oranges and grapefruit may be caused by either of two fungi, Phomopsis citri or Diplodia natalensis. The general appearance of the decay is the same in either case. At first there is a slight softening without discoloration over a small area immediately around the stem or button. This soft area enlarges rapidly and by the time it is an inch in diameter assumes a tan or light-brown color. When the main circular area of rot has included about one-third to one-half the surface of the fruit, fingerlike extensions sometimes spread downward in positions corresponding to the divisions between the segments of the pulp, and a small independent rotted area will regularly appear at the point end, the fungus having reached this region quickly by direct growth through the pith tissue of the core. The fruit may be completely rotted in three or four days from the first appearance of the softening if the temperature is favorable for rapid growth of the particular fungus in question.
>
> Both of these stem-end rot fungi flourish and form spores in dead citrus bark. Phomopsis usually develops most abundantly in recently dead bark of very small twigs and fruit stems; Diplodia occurs most frequently on larger twigs and limbs. During periods of warm, rainy weather the spore crops are produced and disseminated. While all the details have

not been definitely determined, it seems most probable that first infection involves some portion of the stem button of the fruit some time during the growing seasons. Under certain unusual seasonal conditions, usually associated with drought and frequently near the time of maturity, stem-end rot may actively affect the fruit in the groves, causing considerable loss from dropping. Ordinarily little or no evidence of stem-end rot is seen until some time after the fruit is picked. The physiological changes following the separation of the fruit from the tree seem to be favorable for the active growth of both of these stem-end rot fungi and for their more or less rapid extension from the button tissues into the fruit proper. The holding temperature plays an important part in determining the rate of rot for a particular lot of fruit and also whether or not the Phomopsis or the Diplodia rot will prevail in cases of double infection. Diplodia develops best at about 85° F. and Phomopsis at about 75°F. Phomopsis can develop slowly in the 50's, but Diplodia is effectively retarded below 55° F.

Experiments for the control of both types of citrus stem-end rot conducted by the Office of Fruit-Disease Investigations of the Bureau of Plant Industry have followed many lines of attack, some of which have proved successful, some unpromising for commercial control, and some entirely ineffective.[4]

If the fungi causing stem-end rot can be killed while still in the sporadic state, the fruit may be saved. It was with this end in view that 1,100 oranges were treated with high frequency.

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4. Winston, Fulton, and Bowman 1923
"Commercial Control of Citrus Stem-End Rot"
United States Department of Agriculture
Department Circular 293

The experiment was conducted in room 403 of the Mineral Industries Building at Virginia Polytechnic Institute during the month of January 1941. The room is approximately 12 feet wide and 75 feet long, making it a narrow, long room.

The oranges were examined closely, and all fruit showing signs of rot were discarded. The remaining good fruit was divided into two parts: 400 oranges were placed in one end of the long room and left untreated as a check. The remaining 700 were treated by placing them, 3 at a time, between copper plates measuring 4" x 9", and applying a high frequency voltage to the plates.

| Group No. | No. of Oranges in each group | Frequency in Megacycles | Time of Treatment |
|---|---|---|---|
| 1 | 100 | 45 | 15 Sec. |
| 2 | 100 | 45 | 30 " |
| 3 | 100 | 45 | 1 Min. |
| 4 | 100 | 45 | 3 " |
| 5 | 100 | 45 | 5 " |
| 6 | 100 | 45 | 10 " |
| 7* | 100 | 45 | 10 " |

Table I

These treated oranges were placed in the opposite end of the long room from the untreated fruit.

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*This group was first sealed in wax paper and then treated. This procedure was designed to keep the fruit from becoming infected after the treatment.

All of the oranges (treated and untreated) were placed with buttons up in neat rows of 10 oranges to the row. No definite space was left between the oranges, and they often touched. A small space of 4 or 5 inches was left between each group.

The room temperature was observed daily and found to be 23± 3 degrees centigrade at all times.

Every two days all spoiled oranges were taken from each group, counted, and the number recorded. After 30 days the following results had been obtained:

Table II

| Days after Treatment | No. Spoiled | Spoiled to Date (%) |
|---|---|---|
| | CHECK GROUP | |
| 3 | 5 | 1¼ |
| 5 | 1 | 2¼ |
| 7 | 23 | 8 |
| 9 | 9 | 10¼ |
| 11 | 14 | 13 3/4 |
| 13 | 24 | 19 3/4 |
| 15 | 18 | 24¼ |
| 17 | 27 | 31 |
| 19 | 10 | 33½ |
| 21 | 7 | 35¼ |
| 23 | 20 | 40¼ |
| 25 | 23 | 46 |
| 27 | 33 | 54¼ |
| 30 | 54 | 67 3/4 |

Table II continued on the next page--

Table II (continued)--

| | | |
|---|---|---|
| | GROUP NO. 1 | |
| 3 | 3 | 3 |
| 5 | 3 | 6 |
| 7 | 8 | 14 |
| 9 | 1 | 15 |
| 11 | 2 | 17 |
| 13 | 4 | 21 |
| 15 | 3 | 24 |
| 17 | 1 | 25 |
| 19 | 5 | 30 |
| 21 | 1 | 31 |
| 23 | 4 | 35 |
| 25 | 2 | 37 |
| 27 | 1 | 38 |
| 30 | 10 | 48 |
| | GROUP NO. 2 | |
| 3 | 0 | 0 |
| 5 | 1 | 1 |
| 7 | 8 | 9 |
| 9 | 2 | 11 |
| 11 | 3 | 14 |
| 13 | 2 | 16 |
| 15 | 5 | 21 |
| 17 | 1 | 22 |
| 19 | 2 | 24 |
| 21 | 2 | 26 |
| 23 | 3 | 29 |
| 25 | 2 | 31 |
| 27 | 3 | 34 |
| 30 | 4 | 38 |
| | GROUP NO. 3 | |
| 3 | 0 | 0 |
| 5 | 0 | 0 |
| 7 | 9 | 9 |
| 9 | 1 | 10 |
| 11 | 1 | 11 |
| 13 | 5 | 16 |
| 15 | 2 | 18 |
| 17 | 5 | 23 |
| 19 | 3 | 26 |
| 21 | 5 | 31 |
| 23 | 0 | 31 |
| 25 | 4 | 35 |
| 27 | 2 | 37 |
| 30 | 10 | 47 |

Table II--(continued)

| | | |
|---|---|---|
| | GROUP 4 | |
| 3 | 0 | 0 |
| 5 | 1 | 1 |
| 7 | 2 | 3 |
| 9 | 1 | 4 |
| 11 | 0 | 4 |
| 13 | 1 | 5 |
| 15 | 5 | 10 |
| 17 | 3 | 13 |
| 19 | 1 | 14 |
| 21 | 6 | 20 |
| 23 | 3 | 23 |
| 25 | 5 | 28 |
| 27 | 4 | 32 |
| 30 | 8 | 40 |
| | GROUP 5 | |
| 2 | 0 | 0 |
| 4 | 0 | 0 |
| 6 | 4 | 4 |
| 8 | 2 | 6 |
| 10 | 0 | 6 |
| 12 | 6 | 12 |
| 14 | 5 | 17 |
| 16 | 3 | 20 |
| 18 | 3 | 23 |
| 20 | 2 | 25 |
| 22 | 2 | 27 |
| 24 | 0 | 27 |
| 26 | 4 | 31 |
| 28 | 1 | 32 |
| 30 | 5 | 37 |
| | GROUP 6 | |
| 3 | 0 | 0 |
| 5 | 0 | 0 |
| 7 | 4 | 4 |
| 9 | 2 | 6 |
| 11 | 4 | 10 |
| 13 | 4 | 14 |
| 15 | 2 | 16 |
| 17 | 1 | 17 |
| 19 | 14 | 31 |
| 21 | 4 | 35 |
| 23 | 1 | 36 |
| 25 | 4 | 40 |
| 27 | 3 | 43 |
| 30 | 6 | 49 |

Table II--(continued)

| | | GROUP 7 | |
|---|---|---|---|
| 3 | 0 | 0 | |
| 5 | 1 | 1 | |
| 7 | 1 | 2 | |
| 9 | 3 | 5 | |
| 11 | 1 | 6 | |
| 13 | 0 | 6 | |
| 15 | 4 | 10 | |
| 17 | 4 | 14 | |
| 19 | 2 | 16 | |
| 21 | 3 | 19 | |
| 23 | 11 | 30 | |
| 25 | 5 | 35 | |
| 27 | 10 | 45 | |
| 30 | 2 | 47 | |

Table II

Table II shows detailed results of the experiment of treating oranges with high frequency induced electrical energy in an effort to kill the stem-end rot fungi.

A frequency of 45 megacycles per second was used throughout the experiment because it was found that the apparatus delivered more energy to the load at this frequency than at any other without making additions to the oscillator circuit.

By experiment, it was found that the temperature of each orange rose approximately 1°C. per minute of treatment. This rise was measured by inserting the bulb of a thermometer into the orange and taking a reading. The thermometer was then removed and the power turned on the orange. At the end of each minute of treatment, the power was turned off, the thermometer inserted for a

few seconds, a reading taken, the thermometer removed, and the power turned back on. The power had to be turned off to take a reading because the mercury in the thermometer would have been heated by the electric field.

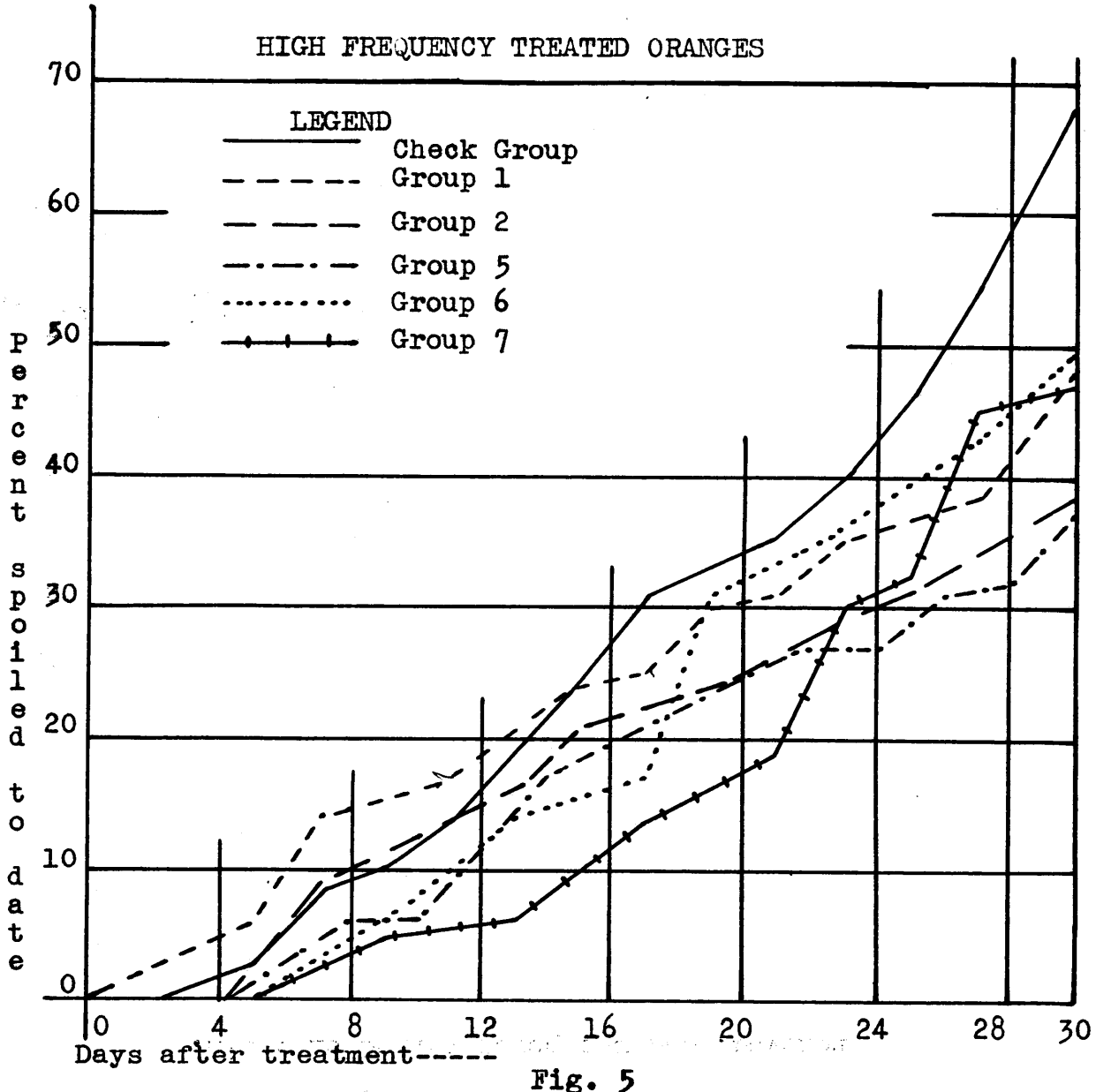


Fig. 5

Graphical representation of some of the results tabulated in Table II.

Discussion and Conclusions:

The results obtained through experiment in this test show only one thing definitely. There was a definite retarding of the growth of the stem-end rot. This effect was slight but unmistakably present. The final percentage of treated fruit spoiled was in every case less than that of the untreated fruit.

An interesting fact worthy of speculation arises from an observation of the curve showing the results of the treatment of the oranges wrapped in wax paper (Curve 7, Fig. 5, Page 19.). Five days passed before any of the fruit spoiled. At the end of 13 days only 6 per cent had spoiled. Suddenly there was swift rise in the number of fruit spoiling each day, and the final figure was one of the highest obtained. Group 6 was treated for the same length of time and at the same frequency. It followed a similar pattern. Possibly this long treatment affected the orange tissues and made them more susceptible to the rot.

The process as used here is of no commercial value. Other methods, such as the Borax treatment, are cheaper, more convenient, and more effective. Perhaps some other frequency or some other arrangement would give better results,

but the experimental cost could be reduced and more definite results obtained by using cultured organisms for the test.

An interesting fact not recorded is that about 99 per cent of the fruit that did spoil spoiled because of stem-end rot.

As stated in the procedure, the oranges were placed between two parallel electrodes (copper plates). They were supported by a thin glass plate, glass being chosen because it has a comparatively low "dielectric hysteresis" loss.

It was noted that when three oranges of different sizes were placed between the plates, the largest of the group seemed to absorb more energy than the other two, that is, its temperature was slightly higher. In some cases where there was too much difference in the sizes of the fruit, it is entirely possible that the smallest orange in the group received too little treatment.

B. Yeast

Remarkable results were obtained at Virginia Polytechnic Institute by the treatment of apple cider.[5] Several bottles of fresh cider were carefully sealed to prevent contamination after the experiment had begun. One-half of these sealed bottles were treated for varying times ranging from two to fifteen minutes. The frequency of these treatments is not known. The treated and untreated cider was then left at ordinary summer room temperature in Blacksburg, Virginia, for six weeks. At the end of this time all bottles were opened and tested for spoilage by tasteing. All bottles that had been treated were found to be unspoiled, while every untreated bottle of cider had begun to ferment.

These results were rather encouraging, so a decision was made to investigate the effects of high frequency exposure on the yeasts respon-

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5. See footnote number 2 listed at the bottom of page 3 for reference to this article. See pages 21 and 22 of this reference.

sible for alcoholic fermentation.*

A saline suspension of Saccharomyces Cerevisciae was used in this experiment. The organisms were treated at 43 megacycles and at 32 megacycles for varying lengths of time and the results noted. The following report was submitted to Dr. F. S. Orcutt of the Biology Department of Virginia Polytechnic Institute by Mr. J. R. Leonards on May 10, 1941:

A heavy suspension of a vigorously growing culture of Saccharomyces cerevisciae in saline solution was counted by the Breed method and diluted with saline to about 3,000 organisms per cc. This suspension was exposed to the high frequency currents for various lengths of time and the number of organisms counted by the plate-count method. The results are given in the following table, the number of colonies being recorded from duplicate plates.

| Test No. | Time of Exposure | Frequency: M. c. s. | No. of Colonies | Dilution | No. of yeasts per ml. |
|---|---|---|---|---|---|
| I | 0 min. | 43 | 1;17 | 1/10 | 90 |
| | 5 " | 43 | 101; | 1/1 | 100 |
| | 10 " | 43 | 96;128 | 1/1 | 110 |
| | 15 " | 43 | 86;123 | 1/1 | 105 |
| II | 0 min. | 43 | 10;11 | 1/10 | 100 |
| | ½ " | 43 | 18;12 | 1/10 | 160 |
| | 1 " | 43 | 11;10 | 1/10 | 100 |
| | 2 " | 43 | 13;12 | 1/10 | 120 |
| | 4 " | 43 | 100;104 | 1/1 | 102 |
| | 7 " | 43 | 61;20 | 1/1 | 40 |
| | 10 " | 43 | 74;87 | 1/1 | 80 |

Table III

*This test was conducted by the Electrical Engineering Department and the Biology Department cooperating. All bacteriological work is credited to Mr. J. R. Leonards, of the Dairy Husbandry department.

All plates in this test were incubated 5 days at 30°C. Other tests were run using a frequency of 32 megacycles per second and exposures up to 20 minutes with about the same results. The temperature of the solution rose from 25°C. to 37°C. during the exposure.

Discussion:

Naturally these results are not very encouraging, but no definite conclusions should be drawn. It may well be that some other frequencies would be very successful. It is a well known fact among bacteriologists that supersonic vibrations have a toxic effect on bacteria. Certainly it would be well to try an electrical bombardment near the effective supersonic frequencies which are just above the audio range. Fabian and Graham obtained their best results at 10 megacycles, but time was not available to build an apparatus capable of producing this frequency.

C. Bacteria

Through the cooperation of the Sanitary Engineering Department, a number of tests were run using Escherichia coli (Bacterium coli) in a standard lactose broth solution. Test tubes of solution containing vigorously growing bacteria were treated at various frequencies from 32 to 55 megacycles and at different lengths of time up to 30 minutes. A transfer was then made to fresh solution and the fresh solution incubated for 24 to 48 hours to obtain a presumptive test. In about 90 per cent of the tests there was positive proof that the bacteria were not all killed. The other 10 per cent might well have been due to experimental error.

It is not considered necessary to give a detailed table of results here because of the completely negative outcome.

This method proves that a completely sterile solution can not be obtained with the frequencies and at the time used. The main disadvantage is that there is no way of knowing if some of the bacteria are destroyed. From

other results obtained, however, it is not reasonable to believe that there was any definite killing effect.

Escherichia coli (Bacterium coli) were used in the experiment performed by Graham and Fabian, reference to which has been made before in this work.

D. Milk

Several bottles of fresh, raw, grade "A", sweet milk were obtained from the Virginia Polytechnic Institute Creamery. A number of bottles were exposed to a high frequency field of 32 megacycles per second for varying lengths of time up to 20 minutes. An equal number of bottles of milk were kept undisturbed. One bottle of pasturized milk was also observed in this test, All of the bottles of milk, treated and untreated raw milk and untreated pasturized milk were exposed to ordinary room temperature (about 26°C.) for 24 hours. At the end of this period all of the milk had spoiled except the pasturized bottle. The high frequency treatment apparently did not delay the spoiling any.

A period of 20 minutes was the maximum used in this test, because one bottle was treated for 25 minutes and was made to boil in that length of time.

An interesting fact noted was that the cream rose more slowly on the treated milk. It is not known to the experimenter if this was due to different characteristics of the milk used, or if the high frequency exposure tended to break up the large fat globules so that the fat rose to the top more slowly.

# VI

# GENERAL DISCUSSION OF RESULTS AND CONCLUSIONS

The results of the experiments run are briefly stated as follows:

Induced electrical energy of from 40 to 55 megacycles per second does retard the growth of stem-end rot in oranges slightly, but this is not a very effective means to that end. This statement should not be construed to mean that other frequencies not mentioned may not be just as or more effective. The frequencies mentioned are the only ones used.

Furthermore, it is not possible to kill yeast or Escherichia coli (Bacterium coli) by the methods employed in this test.

It seems possible that some other frequencies might give more satisfactory results. It is suggested a frequency of about 30,000 cycles per second be tried if this project is to be continued. A high voltage, several hundred volts, should be used on the plates.

There are few facts to support this statement and almost no reason to believe that positive results would be obtained, except that

supersonic frequencies near this are known to be effective. This, of course, is merely a guess, but it seems to be the next most logical step. It is very highly probable that no positive results can be obtained at any frequency, but the process would be so useful that the field is well worth exploring.

# VII

## EPITOME

This experiment was performed in an effort to kill certain types of micro-organisms including yeasts, orange rot fungi, bacteria common to the human intestines, and bacteria causing milk spoilage.

The different organisms were taken in some favorable medium and placed between two parallel flat copper plates. An alternating voltage of from 32 to 55 megacycles was then applied to the plates. This voltage induced high frequency circulating electric currents within the medium and, necessarily, within the organisms themselves. It was the experimenter's hope that these circulating currents would prove distructive to the organisms.

The orange rot fungi were affected slightly, but for all other organisms tried, the results were so small as to be almost entirely masked in experimental error.

No provisions were made to keep the media containing the organisms cool. As a result, the treatment could be given for only short periods of time. If longer times had been used, the media would have become too hot.

In this case, the organisms may have been destroyed purely by heat. If the killing effect is not to be developed in a very short period of time, some provisions must be made to keep the media cool.

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