THE INFLUENCE OF VARIOUS FACTORS ON ULTRAVIOLET LIGHT STERILIZATION OF WATER

By

PAUL E. DEGURSE

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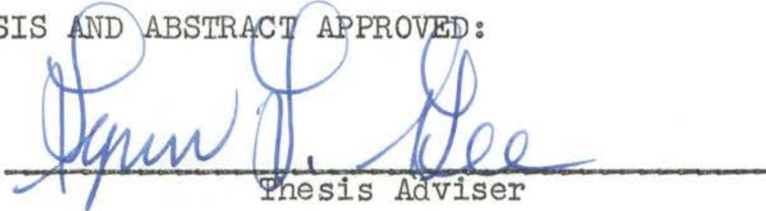
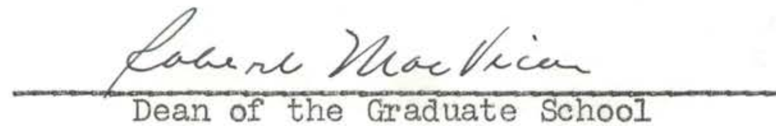


Thesis Adviser

Faculty Representative



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INTRODUCTION

The disinfection of small water supplies of the farm, rural schools, and small communities presents a problem that has not been completely solved by chemical treatments. Chlorinating devices which adequately treat small water supplies require frequent attention. Automatic chlorinators are not economically feasible for these small water supplies. The recent development of low temperature ultraviolet light tubes which have a high output of germicidal energy has renewed interest in the utilization of this method of sterilizing water supplies on which chemical treatment is not economically feasible or is otherwise undesirable (Gilcreas and De Lalla, 1953). Several companies are at present producing ultraviolet light sterilizers which are advertised as effectively treating water supplies varying in capacity from a few quarts per minute to hundreds of gallons per hour.

The effective application of ultraviolet light to the sterilization of water is dependent upon several physical and biological factors. Among these are the wavelength of the light, intensity of the germicidal energy, exposure time, and the resistance of the microorganisms. The intensity of the germicidal energy varies with the age of the ultraviolet light emitting tube, and the specific absorption of the water. The output of an ultraviolet lamp drops to approximately 90 per cent of the original after

100 hours operation. The intensity decreases more gradually to approximately 50 per cent at the end of 5000 hours operation time (Cortelyou,et al., 1954a). The specific absorption of germicidal energy by natural waters has been shown to vary over a wide range. Certain waters absorb 90 per cent of incident germicidal energy in approximately 35 inches of water, while others absorb the same amount in approximately 5 inches (Luckiesh, et al., 1944). The most effective wavelength and exposure time for the destruction of coliform bacteria in clear water have been well established (Wyckoff, 1931; Gates, 1929; Luckiesh, et al., 1944). Different species and strains of microorganisms are known to vary in their resistance to the effects of ultraviolet light irradiation (Rentschler, et al., 1941; Hollaender and Claus, 1935). Environmental conditions before and after irradiation influence the response of bacteria to ultraviolet light. The presence of high concentrations of sodium or potassium salts in the growth medium increases the resistance of bacteria to ultraviolet light irradiation (Durham, 1954). Incubating ultraviolet irradiated bacteria in salt solutions at 30 to 45° centigrade increases survival (Roberts and Aldous, 1949). Photoreactivation, by visable light, of ultraviolet irradiated bacteria has been demonstrated (Kelner, 1949).

There is considerable variation in respect to ions in solution and turbidity in natural water supplies. Aside from the effect of iron upon the transmittance of ultraviolet

light through water (Luckiesh and Holliday, 1944), there is very little information in the literature concerning the influence of common mineral ions upon ultraviolet light sterilization of water. So far as is known, turbidities which were investigated in relation to ultraviolet sterilization of water were those created by such materials as diatomaceous or Fuller's earth. Since a good deal of turbidity in many natural waters is caused by clay of varying particle size, it was deemed essential that an investigation be initiated which would provide information in respect to this variable. This experimental work was initiated to obtain information on the influence of ions in natural waters upon the biological response of bacteria to ultraviolet light, and the influence of different size clay particles on the ultraviolet treatment of water.

Studies were also made on the influence of photoreactivation on survival rates of small populations of ultraviolet irradiated bacteria.

LITERATURE REVIEW

As early as 1909 experimental work was conducted in France to test the feasibility of ultraviolet light sterilization of water (Grant, 1910). The earliest record of an installation of apparatus for ultraviolet light sterilization of a municipal water supply in the United States was in 1916 at Henderson, Kentucky (Smith, 1917). This worker reported the effectiveness of the plant at Henderson to be adequate. However, this plant was abandoned some time between 1923 and 1924 (Baker, 1948). Blocher (1929) described an ultraviolet system installed at Berea, Ohio, which he reported effectively treated 84,000 gallons of filtered water per hour. Perkins and Welch (1930) reported effective disinfection of water with a "C" carbon arc. These workers reported a residue of resistant forms which was not reducible within practical commercial limits. Baker (1948) states that all the municipal installations in this country have been abandoned and but few industrial-communal plants are in operation. He also states that no record could be found of plants still in operation in Europe. The reasons given for replacing the ultraviolet light treatment plants were high operation costs and the belief of state officials that chlorination would be of greater efficiency (Baker, 1948).

The production of low-pressure mercury tubes made from ultraviolet light transmitting glass and which operate with

good efficiency at 18 to 25° centigrade has stimulated a renewed interest in the application of ultraviolet energy to the sterilization of water. The greatest interest being in the application of this method to the treatment of small water supplies (Gilcreas and De Lalla, 1953). Luckiesh, et al. (1944) stated that the energy from one 30 watt ultraviolet lamp will destroy 99 per cent of *Escherichia coli* in 7,000 gallons of water per hour. These workers suggest that the sterilizer be designed so that 90 per cent of the energy be absorbed in the water, thus preventing energy from being wasted on the walls of the treatment chamber.

Luckiesh and Holliday (1944) determined the lethal dose of ultraviolet energy for *Escherichia coli* in water to be approximately 40 microwatt minutes/cm^2 (2.4 x 10^4 ergs/cm^2). Rentschler, et al (1941) report that 10 exposures of 1 second duration at 220 microwatts/cm^2 were sufficient for 99 per cent kill of *Escherichia coli* on the surface of agar media. These workers demonstrated that a decrease in intensity could be overcome by an equivalent increase in exposure time. Coblents and Fulton (1924) using low light intensities found that the law of reciprocity was not strictly followed. With a 50-fold reduction in light intensity, the exposure time had to be increased by a factor of 75 to obtain comparable lethality. Hollaender (1943) demonstrated that the energy necessary for a 50 per cent kill of *Escherichia coli* at 2650 Å was

10^3 ergs/sec./cm^2. The most effective wavelength of ultraviolet light for destruction of bacteria is near 2650 Å (Gates, 1929; Duggar and Hollaender, 1934; Hollaender and Claus, 1935; Wyckoff, 1931; Hollaender, 1943). The energy necessary to kill 50 per cent of *Escherichia coli* at a wavelength of 2536 Å was shown to be 200 ergs/mm^2 and that at 2652 Å was 110 ergs/mm^2 (Wyckoff, 1931).

The temperature coefficient of the bactericidal action of ultraviolet light in the spectra below 3000 Å was shown to be 1.1 (Hollaender, 1943). Smith and Perry (1941) during an investigation of the use of ultraviolet rays in sterilizing vegetable hydrocooler water, noted that temperature variation from 1 to 40° centigrade was not a factor in determining the effectiveness of kill. The energy output of the lamp may be decreased as much as 75 per cent by immersing it in water at temperatures of 5° centigrade (Cortelyou, et al., 1954b).

The treatment of bacterial cells after ultraviolet irradiation greatly influences the survival rates. The reduction of the lethal action of ultraviolet light by exposing the irradiated cells to visible light has been demonstrated (Kelner, 1949). The action spectra for this "photoreactivation" has been established to be below 5000 Å with a peak of function at 3750 Å (Kelner, 1951). Roberts and Aldous (1949) noted a recovery of irradiated

cells of *Escherichia coli* when they were incubated at 37° centigrade in a phosphate buffer containing ammonium chloride and magnesium sulfate. This recovery apparently reached a maximum after 5 hours. Cells placed on synthetic media resulted in higher survival rates than those plated on standard nutrient agar. A reduction in the concentration of agar used in the plating media resulted in an increased rate of survival. Cells held at 5° centigrade for 5 hours showed no significant variation from the controls. Anderson (1951) verified the work of Roberts and Aldous and stated that there was a significant increase in survival rates with a 10° rise in temperature from 30 to 40° centigrade. Heat reactivation of ultraviolet light irradiated *Escherichia coli* strain B follows much the same order as light reactivation. Strain B/r is not as sensitive to heat reactivation as is strain B (Anderson, 1951).

Claus (1933) demonstrated that the lethal effect of X-ray radiation of *Escherichia coli* is enhanced by salts of heavy metals. Durham (1954) demonstrated that the presence of sodium or potassium salts in concentrations of 0.5 to 4.0 per cent in the culture media in which the cells were grown greatly increased the resistance of *Escherichia coli* strain B and strain B/r, to ultraviolet light irradiation.

Luckiesh, et al.(1944) and Luckiesh and Holliday (1944) reported that iron in colloidal solution influences the transmittance of ultraviolet light in water. These workers stated that the light transmittance through 5 inches of water

may be reduced from 93 to 7 per cent by as little as 1 ppm of iron in solution in the form of $FeCl_3$.

Turbidities in excess of 75 ppm were sufficient to reduce the sterilizing action of a 30 watt ultraviolet lamp operating through a distance of 3/8 inches of water with an exposure time of 4.2 seconds (Gilcreas and De Lalla, 1953). The turbidity in this instance was caused by diatomaceous earth. Organic turbidity such as that caused by skim milk at the rate of 30 ppm in water protected bacteria to the same degree as 100 ppm of diatomaceous earth (Gilcreas and De Lalla, 1953).

METHODS AND MATERIALS

I. General Procedures

In order to study the influence of certain ions in solution and clay turbidities of different particle size on the sterilization of water, it was necessary to devise a standardized procedure for irradiating and sampling the bacteria in the water. Since the inactivation of bacteria exposed to ultraviolet light is exponential between 20 and 80 per cent killing (Hollaender, 1943), a survival rate between 40 and 60 per cent was selected for control treatments. The 40 to 60 per cent survival rate was obtained by fixing the exposure time and varying the intensity of the ultraviolet light by changing the distance between the light source and the cells. Sterile distilled water buffered to pH 7.2 was used in making the solutions of various ions or clay suspensions. Water with ions in solution or with suspensions of different sized clay particles was inoculated with a standard inoculum of _Escherichia coli_ strain B to give a final concentration of 1 to 2 million cells per ml. The cell suspensions were then irradiated by passing the water at a constant rate of flow through a crystal quartz tube mounted horizontally in front of a 4 watt germicidal lamp. Samples of non-irradiated water were taken from the inflow end of the tube and irradiated samples were taken from the outflow end. Five irradiated and five non-irradiated samples were

taken for each treatment. Total bacterial counts were made by plating the samples in nutrient agar and incubating at 37° centigrade for 48 hours.

The ends of the quartz tube were masked to provide an exposure length of 18 cm. The flow rate was maintained at 1 liter per minute for all treatments. The center of the quartz tube was 3 cm and the ends 9.5 cm from the light source. By selecting points along the base of a triangle in which the quartz tube served as the base line and the lamp as the apex, several measurements were made to determine the ultraviolet light energy at calculated distances from the lamp. From these measurements, the average intensity transmitted through the quartz tube containing clear water was calculated to be approximately 540 microwatts/cm^2. At a flow rate of 1 liter per minute, the cells in suspension were exposed approximately 0.5 seconds, and therefore received approximately 3×10^3 ergs/cm^2/sec.

II. Description of Apparatus

The ultraviolet light source used in this investigation was a General Electric Company, 4 watt, germicidal lamp. The lamp was seated in the "head" of a small ultraviolet-light water sterilizer (Cortelyou, et al., 1954a). The sterilizer head and bracket were mounted in the rear of a wooden box the dimensions of which were 24 by 23 by 14 cm (Figure 1). The mounting was so arranged that the lamp was inside, perpendicular to the bottom, and equidistant from the sides of the box. The top of the box was flush with the sterilizer head, and a window was cut to facilitate measurements of light intensity. Parallel slots were cut in each side of the box. Sliding panels were placed over the slots and a hole coinciding with the slots drilled in each panel. A crystal quartz tube with an inside diameter of 8 mm and an outside diameter of 10 mm was passed through the holes in the panels. With this arrangement the quartz tube was in a horizontal position in front of the lamp. The distance between the lamp and the quartz tube could be varied by moving the sliding panels.

Water was supplied to the quartz tube through glass tubing from a 17 liter carboy mounted on a stand 2½ feet above the quartz tube (Figure 2). Constant pressure was maintained by the use of a constant flow siphon. The flow rate was measured by a flowrator and controlled by means

Figure 1

Diagram of Lamp and Quartz Tube Mount

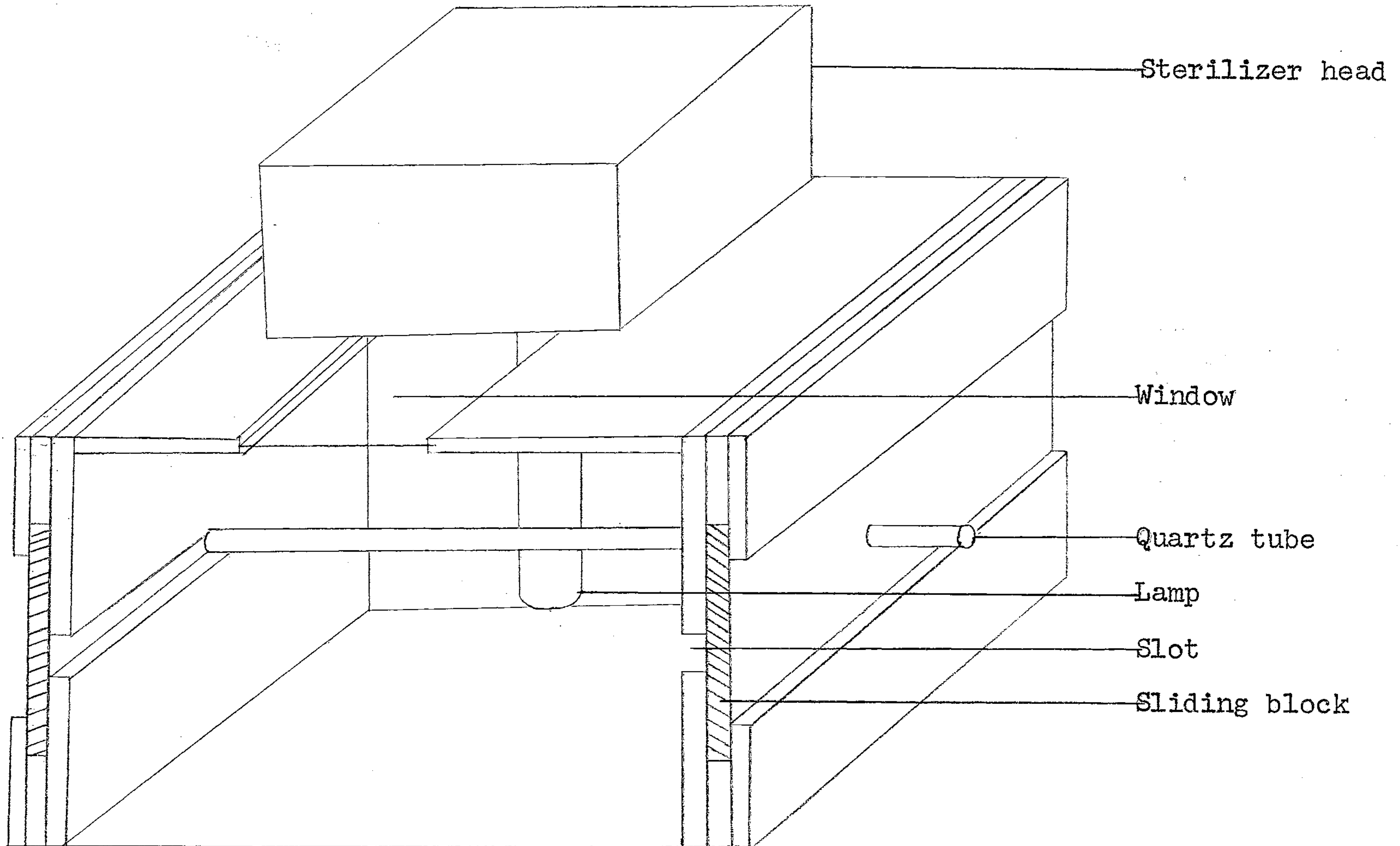
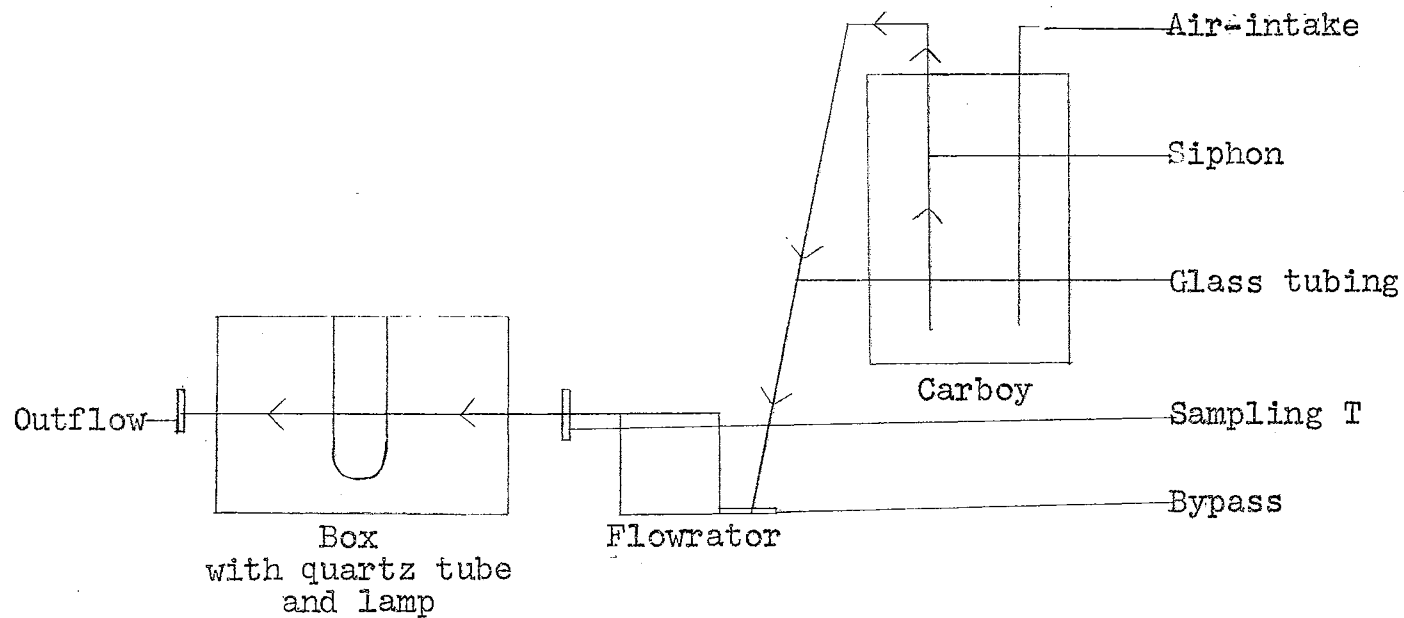


Figure 2

Diagram of Treatment System



of a needle valve attached to the outflow end of the quartz tube. An outlet was provided at the bottom of the flowrator which served as a bypass and allowed for the application of negative pressure to start the flow through the siphon. When this bypass was closed water would flow through the flowrator and the quartz tube. A sampling bypass was inserted between the flowrator and the quartz tube from which non-irradiated samples were obtained.

The front end of the box in which the lamp was mounted was left open in order that measurements could be made of the ultraviolet light transmitted through the water under investigation. Ultraviolet light energies were measured by means of a light meter equipped with a Luckiesh-Taylor germicidal attachment.[1] In order that only the light which passed through the quartz tube and the water within the tube be measured, a cardboard shield was placed above and below the tube. The light meter was mounted on a sliding block of wood which was held by means of a channel to a larger block (Figure 3). A centimeter scale was fixed to the larger block and a needle pointer set in the sliding block so that reproducible measurements of ultraviolet light could be made at desired distances from the source.

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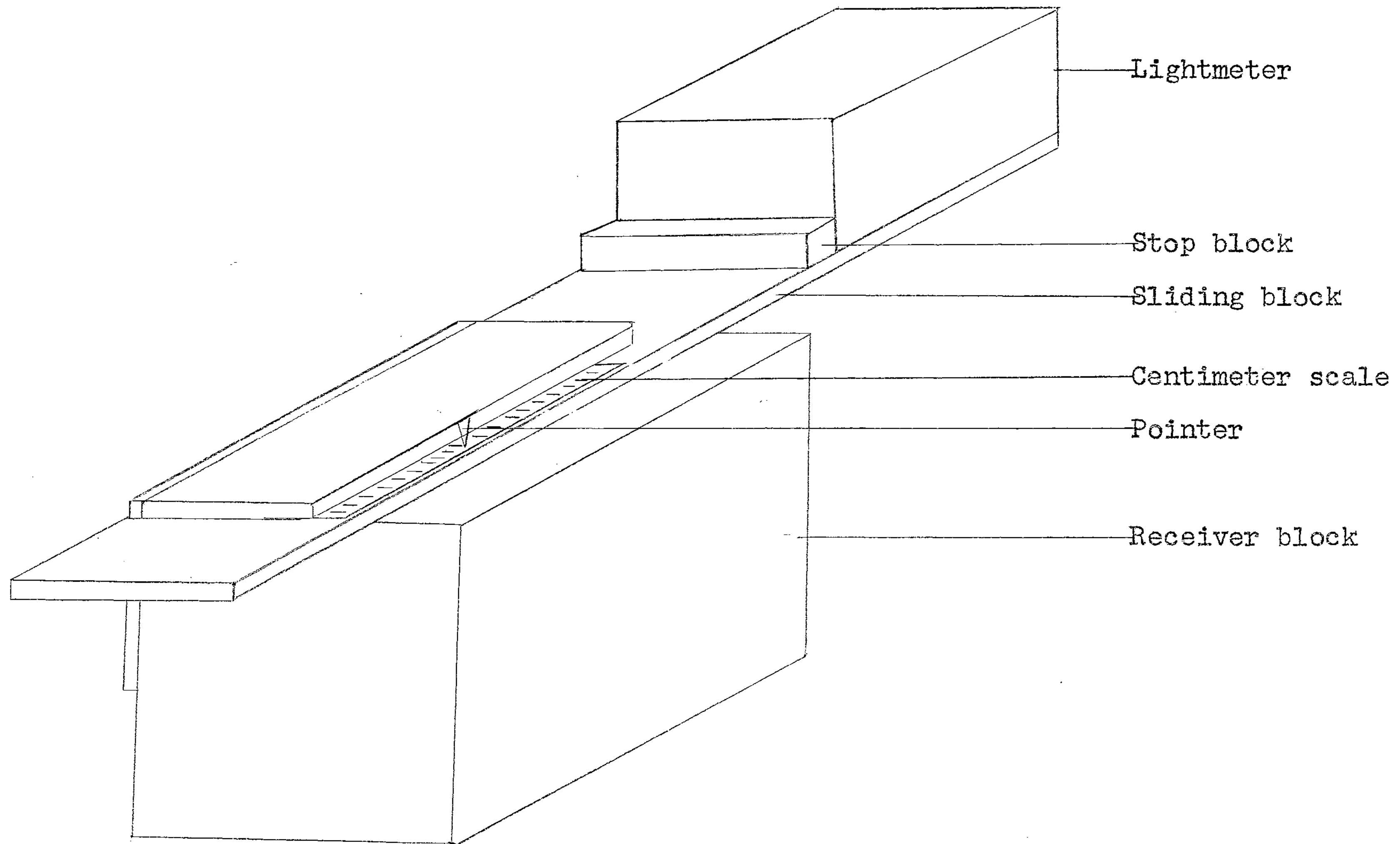
[1]General Electric Company, Nela Park, Cleveland, Ohio.

Figure 3

Diagram of Lightmeter Mount



III. Culture

The microorganism used throughout this work was *Escherichia coli* strain B. The culture was maintained on nutrient agar slants and stored at 5° centigrade. Transfers were made once each month to fresh slants, incubated 48 hours at 37° centigrade, and returned to storage until used. Cultures for experimentation were prepared by transfer from agar slants to 30 milliliters of nutrient broth in 250 ml Erlenmyer flasks. The broth cultures were incubated 24 hours at 35° centigrade with constant shaking on a rotary shaking machine operating at approximately 180 rpm. This procedure produced cultures with a constant population of approximately 5×10^9 viable cells per milliliter. Standard cell suspensions for inoculating water were prepared by centrifuging the cells from a measured amount of the broth culture. These cells were washed and resuspended in 0.02 molar sodium or potassium phosphate buffer.

IV. Media

Nutrient broth used to culture the bacteria and as a base for all nutrient agar was of the following composition: 3 grams yeast extract (Difco) and 5 grams peptone (Lewis) per liter of distilled water. All media were adjusted to pH 6.8 by the use of 1.0 N NaOH and dispensed in tubes or flasks in which they were sterilized at 120° centigrade for 20 minutes in the autoclave.

Agar medium was made by adding 15 grams of agar-agar (Difco) per liter of nutrient broth. The nutrient agar for plating was prepared just prior to the time it was to be used, dispensed in 1 liter flasks, sterilized and placed in a water bath at 45° centigrade until used.

V. Preparation of Clay

Two clay fractions widely separated in respect to particle size were obtained by sedimentation procedures from clay dispersed in water. The two clay fractions selected were made up of particles which had sedimentation rates equivalent to spherical particles 1 to 2 µ and 0.1 to 0.2 µ in diameter as determined by the application of Stoke's law for rates of settling of spherical particles. Actual determination of particle size of the clay could not be made by this method since clay particles are not spherical.

Dispersal of clay in water was accomplished by adding approximately 500 grams of clay to 2 liters of water, adding an excess of sodium oxalate and adjusting the pH to between 8.5 and 9.0 with 1.0 N NaOH (Puri, 1949). This mixture was shaken intermittently for 1 to 2 hours and allowed to stand a few minutes to permit large particles to settle out. The supernatant was decanted into a blendor and agitated 1/2 hour. The suspension was then transferred to 1 liter graduate cylinders and allowed to stand 1 hour. The material remaining in suspension at

the end of this time was decanted and centrifuged at 112 times gravity. The clay which settled out at this force will be referred to as the 1 to 2 µ fraction. This fraction of clay was resuspended in a dilute sodium hydroxide solution. The supernatant was concentrated by boiling to 1 liter and centrifuged at 1790 times gravity for 1 hour. The material which remained in suspension was discarded. The clay which settled out in this treatment was resuspended in dilute sodium hydroxide and again centrifuged at 1790 times gravity for 15 minutes. The sediment was discarded and the supernatent centrifuged for 1 hour at 1790 times gravity. The particles which settled out in the last operation will be referred to as the 0.1 to 0.2 µ fraction. This sediment was resuspended in the same manner as the 1 to 2 µ fraction. The clay suspensions were sterilized in the autoclave at 120° centigrade for 30 minutes in tightly stoppered flasks and allowed to cool. The amount of clay in each suspension was determined by weighing aliquots which had been evaporated to dryness in an electric oven at 105° centigrade. Frequent redeterminations were made in order that changes in weight due to the loss of water would be detected.

When clay turbidities of various concentrations were to be tested for light absorption or influence on ultraviolet sterilization, a calculated amount of clay was added from the clay suspensions to buffered distilled

water. The buffer used for all clay experiments was sodium phosphate.

VI. Procedure for Cations in Solution

Control cells were treated with ultraviolet light in buffered distilled water. The total salt concentration of such water was approximately 280 ppm. Cells to be tested for response to ions were placed in buffered distilled water to which had been added the chloride salt of the particular cation under study. The cations and concentration of each tested were: Na-500 ppm, Mg-500 ppm, K-50 ppm, and Fe-5 ppm. The control and cation waters were inoculated with enough standard cell suspension to give final concentrations of 1 to 2 million bacteria per ml. After inoculation, both the control and the water containing the various cations were placed in the refrigerator at 10° centigrade for 8 to 10 hours. At the end of this period the water was brought to room temperature and placed in the carboy reservoir of the system previously described. Five samples of irradiated and non-irradiated water were taken at random for both the control and cation treatments. Each sample was diluted with sterile buffered distilled water to a dilution which would contain more than 30 but less than 300 bacteria per ml. One ml of this dilution was plated in nutrient agar in each of 3 to 5 replicate plates. Counts were made of total colonies after 48 hours incubation at 37° centigrade.

VII. Procedure with Turbidities

Fifteen liters of distilled water buffered with sodium phosphate to pH 7.2 were placed in the reservoir of the treatment system and inoculated with enough standard cell suspension to give concentrations between 1 and 2 million bacteria per ml. Five liters of this water were allowed to flow through the apparatus and 5 random samples taken as non-turbid controls. The remaining 10 liters in the reservoir were made to 50 ppm clay suspension by the addition of an appropriate amount of one of the sterile stock suspensions. Five more liters were allowed to pass through the apparatus and 5 random samples taken as the 50 ppm treatment. The 5 liters remaining in the reservoir were made up to 100 ppm with an appropriate amount of the same stock suspension. This liquid was allowed to flow through the apparatus and five random samples taken as the 100 ppm treatment. Other experiments were performed in which the same procedure was used except that the reservoir water was made up to 100 and then 150 ppm clay. Non-irradiated controls were taken at random from the entire 15 liters of water. Five replicate plates were made of each sample, after suitable dilution in sterile buffered distilled water.

In order to obtain information on the possibility that clay turbidity may protect bacterial cells from ultraviolet irradiation other than by absorption of light,

experiments were performed with bacteria in clear water exposed to intensities of ultraviolet light reduced to the amount transmitted by 100 and 150 ppm of each clay fraction. This was accomplished by moving the quartz tube containing clear water away from the light source until the ultraviolet light transmitted was the same as that transmitted by the turbid water.

VIII. Measurements of Transmittance of Light Through Clay Suspensions

The transmittance of light through water containing 50 ppm of each of the clay fractions was determined in the visible and ultraviolet light spectra between 650 and 350 and at 254 mμ. The measurements in the visible spectrum were made at intervals of 25 mμ. The transmittance of visible light was measured by a photoelectric spectrophotometer. Ultraviolet light was measured by the use of the instrument described previously.

IX. Photoreactivation Procedure

Bacteria which had been irradiated with ultraviolet light in buffered distilled water were exposed to high intensities of visible light for 45 minutes to 1 hour. The temperature of the cell suspensions was maintained between 30 and 34° centigrade during this treatment. Controls from the same irradiated population were kept in the dark the same time and temperature as the light

treatments. At the end of the exposure period all samples were placed in the refrigerator at 10° centigrade until plated. All samples were plated in nutrient agar with 3 to 4 replicate plates for each sample. The plates were incubated 48 hours at 37° centigrade and total counts made of viable cells.

The total number of cells in the suspensions before irradiation ranged between 1 and 2 million per ml. Approximately 50 per cent of the cells were killed by the ultraviolet light treatment.

The light source for photoreactivation was a General Electric Company, number 1 photoflood lamp. The light was filtered through a 2 per cent aqueous copper chloride solution to remove all infrared light. The light and filter were mounted in a water bath made from a glass cylinder which was 6 inches in diameter. The cell suspensions to be treated were placed in sterile test tubes fastened to the outside of the water bath by means of large rubber bands. The upper 1/8 of the test tubes was above the top of the cylinder. The tubes were filled to a level which would keep the cell suspensions below the top of the cylinder. The water bath was placed in a sink and supplied with water at 30 to 34° centigrade from the tap. The water was allowed to flow continuously, and overflowed onto the lower part of the test tubes to keep them at the same temperature as the bath. A water

bath without the light and filter was used for the dark controls. The filter was made by placing an 800 ml beaker inside a 1 liter beaker and filling the interspace with the copper chloride solution. The lamp was placed inside the 800 ml beaker. The lamp and filter were held in place in the water bath by means of ring clamps.

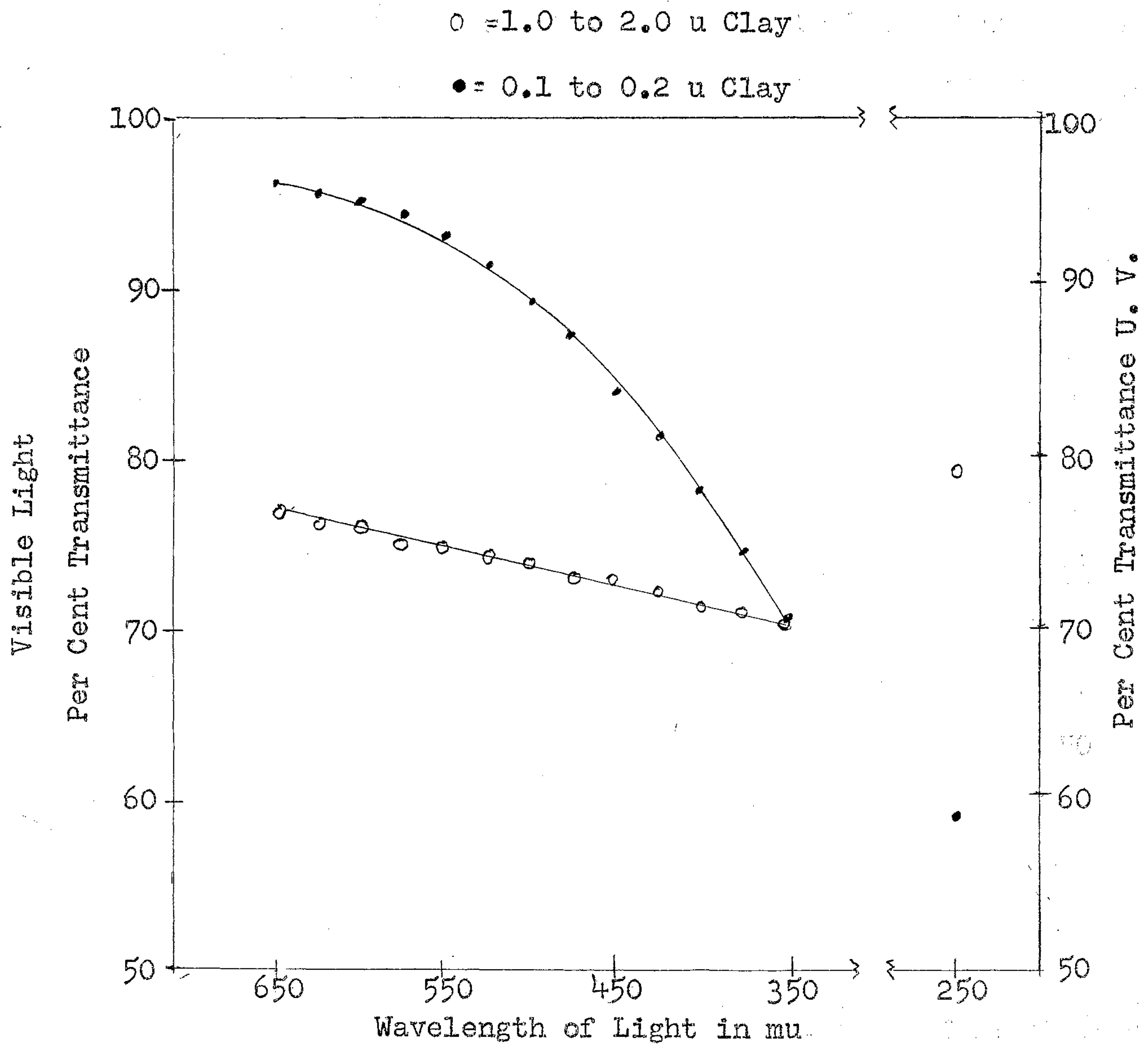
RESULTS

The Influence of Particle Size of Clay Suspensions on the Transmittance of Visible and Ultraviolet Light

The transmittance of visible light of the longer wavelengths by the two clay fractions in suspension in water differs greatly (Figure 4). This difference, 19 per cent at 650 mμ, becomes less as the wavelength of light decreases until at 350 mμ the transmittance is the same for the two clay fractions. The 1.0 to 2.0 μ clay fraction shows a gradual decrease in transmittance, from 77 per cent to 70 per cent, as the wavelength is decreased from 650 to 350 mμ. The 0.1 to 0.2 μ clay fraction, on the other hand, shows a drop in transmittance from 96 per cent to 70 per cent over the same spectrum. At a wavelength of 254 mμ 20 per cent less light is transmitted by the suspension made up of particles from the 0.1 to 0.2 μ fraction than by the suspension made up of particles from the 1 to 2 μ clay fraction. There is an apparent cross-over of the transmittance curves of these clay fractions as the wavelength of light decreases from 650 to 254 mμ. This indicates that measurements of turbidity by visible light transmittance cannot be used as a criterion for ultraviolet light treatment of water.

Figure 4

Per Cent Light of Different Wavelengths Transmitted by 50 ppm of Each of the Clay Suspensions



The Influence of Clay upon Survival Rates of Bacteria Irradiated in Water

The survival rates of bacteria irradiated in water made turbid with clay of each of the fractions are significantly higher than the survival rates of bacteria irradiated in clear water in all treatments except those containing 50 ppm of the 1 to 2 μ clay fraction (Tables 1, 2 and 3). No significant difference in survival rates could be detected between the two suspensions at 50 ppm. The difference between the survival rates of cells irradiated in suspensions of 100 ppm of the 1 to 2 μ clay fraction and the 0.1 to 0.2 μ clay fraction is highly significant. The difference between survival rates of cells irradiated in suspensions of 150 ppm of each of the clay fractions is also significant.

The Influence of Cations on Survival of Irradiated Bacteria

The survival rates of *Escherichia coli* allowed to stand pre-irradiation at 10° centigrade for 8 to 10 hours in buffered water and buffered water containing the chloride salt of various cations were not significantly different in any of the treatments except those irradiated in the presence of iron (Tables 5, 6, 7, 8 and 9). Iron at 5 ppm absorbed 37 per cent of the incident ultraviolet light and cells irradiated in 5 ppm iron survived at a significantly higher rate than those of the

controls. *Escherichia coli* was irradiated in buffered water free from iron with the transmitted ultraviolet light reduced to the amount transmitted by buffered water containing 5 ppm iron. The results obtained from this treatment indicate that the increase of survival rates of cells irradiated in water containing 5 ppm of iron was due to the absorption of ultraviolet light (Table 9).

The possibility was considered that the slight upward trend in survival rates of the bacteria irradiated in the presence of 500 ppm of the chloride salt of the various cations was due to osmotic pressure differences. Experiments were performed in which two wide ranges of total salt concentrations were used. The results indicate that osmotic pressure as produced by these salt concentrations, did not influence ultraviolet irradiation effects on *Escherichia coli* in water (Table 10).

The Influence of Photoreactivation on Small Populations of Irradiated Bacteria

There is a good deal of variation in the data obtained from the experiments on photoreactivation (Table 11). The data obtained in the first two experiments indicate an increase of survival rates of irradiated cells which were exposed to visible light. The last two experiments indicate no change in survival rates. When the combined data were analyzed, no significance could be attributed to the visible light treatment.

Table 1

Survival of *Escherichia coli* irradiated in clear and turbid water

Number of Cells x 10^4

	Control (No turbidity)		50 ppm clay 1-2 u		50 ppm clay .1-.2 u	
	Non-Irradiated	Irradiated	Non-Irradiated	Irradiated	Non-Irradiated	Irradiated
Test No. 1	124*	71	134	86	138	93
2	87	53	103	68	89	65
3	94	54	91	56	98	73
4	139	90	142	87	151	99
5	124	75	125	88	112	84
6	113	52	91	60	106	69
Average	113	66	115	74	112	81
Per cent Survival	58.0		65.2		69.5**	

* All figures are the average of 5 replicate plates from each of 5 random samples.

** Significantly greater than the control at the 5% level.

Table 2

Survival of *Escherichia coli* irradiated in clear and turbid water

Number of Cells x 10^4

	Control (Zero turbidity)		100 ppm clay 1-2 u		100 ppm clay .1-.2 u	
	Non-Irradiated	Irradiated	Non-Irradiated	Irradiated	Non-Irradiated	Irradiated
Test No. 1	143*	90	144	94	149	125
2	122	88	122	98	113	95
3	84	34	82	41	82	59
4	94	30	95	41	97	64
5	178	87	188	110	176	133
6	197	123	192	142	204	165
Average	136	75	139	87	134	103
Per cent Survival	55		64**		77**	

* All figures are the average of 5 replicate plates from each of 5 random samples.

** Significantly greater than the control at the 1% level and the difference between these is significant at the 1% level.

Table 3

Survival of *Escherichia coli* irradiated in clear and turbid water

Number of Cells x 10^4

	Control (Zero turbidity)		150 ppm clay 1-2 u		150 ppm clay .1-.2 u	
	Non-Irradiated	Irradiated	Non-Irradiated	Irradiated	Non-Irradiated	Irradiated
Test No. 1	83*	34	83	47	82	58
2	198	126	191	152	176	144
3	178	83	189	112	205	176
4	180	102	182	122	175	144
Average	159	86	162	109	159	131
Per cent Survival	54		67**		81**	

* All figures are the average of 5 replicate plates from each of 5 random samples.

** Significantly greater than the control at the 1% level and the difference between these is significant at the 1% level.

Table 4

Numbers of cells surviving ultraviolet irradiation in clay suspensions and with light intensity reduced to correspond to that transmitted by the clay suspensions

	100 ppm 1 - 2 µ Clay		100 ppm 0.1 - 0.2 µ Clay		150 ppm 1 - 2 µ Clay		150 ppm 0.1 - 0.2 µ Clay	
Test No.	1	2	3	4	5	6	7	8
Rep.								
1	140*	127	135	164	157	118	179	139
2	136	124	141	176	161	118	174	154
3	142	133	139	161	148	116	168	153
4	142	118	132	174	141	98	184	143
5	153	129	136	175	145	112	187	134
Means	142.6	126.2	136.6	170.0	150.4	112.4	178.4	144.6

	Light Reduced Equivalent to 100 ppm 1 - 2 µ Clay		Light Reduced Equivalent to 100 ppm 0.1 - 0.2 µ Clay		Light Reduced Equivalent to 150 ppm 1 - 2 µ Clay		Light Reduced Equivalent to 150 ppm 0.1 - 0.2 µ Clay	
Test No.	1	2	3	4	5	6	7	8
Rep.								
1	153	124	142	174	151	108	174	152
2	153	121	140	175	149	116	199	157
3	141	130	151	169	155	114	189	151
4	132	123	137	160	148	104	192	155
5	143	107	138	165	157	111	189	142
Means	144.4	121.0	141.6	168.6	152.0	110.6	188.6	151.4

*Each figure represents the average from 5 replicate plates.

Table 5

Survival of *Escherichia coli* irradiated after standing in buffered water and buffered water containing 500 ppm Na as NaCl

Number of Cells x 10^4

	Buffered Water		500 ppm Na	
	Non-irradiated	Irradiated	Non-irradiated	Irradiated
Test No. 1	122*	51	124	53
2	111	50	107	59
3	149	78	150	92
4	190	113	194	114
Average	144	74	145	79
Per cent Survival	51		55	

* All figures are the average of 3 replicate plates from 5 random samples.

Table 6

Survival of *Escherichia coli* irradiated after standing in buffered water and buffered water containing 50 ppm K as KCl

Number of Cells x 10^4

	Control		50 ppm K	
	Non-irradiated	Irradiated	Non-irradiated	Irradiated
Test No. 1	121*	37	154	56
2	211	91	171	67
Average	161	64	162	62
Per cent Survival	38		38	

* All figures are the average of 3 replicate plates from each of 5 random samples.

Table 7

Survival of *Escherichia coli* irradiated after standing in buffered water and buffered water containing 500 ppm Ca as $CaCl_2$

Number of Cells x 10^4

	Control		500 ppm Ca	
	Non-irradiated	Irradiated	Non-irradiated	Irradiated
Test No.				
1	124*	53	118	54
2	219	87	243	114
Average	172	70	180	83
Per cent Survival	41		46	

* All figures are the average of 3 replicate plates from each of 5 random samples.

Table 8

Survival of *Escherichia coli* irradiated after standing in buffered water and buffered water containing 500 ppm Mg as $MgCl_2$

Number of Cells x 10^4

	Control		500 ppm Mg	
	Non-irradiated	Irradiated	Non-irradiated	Irradiated
Test No.				
1	216*	86	217	63
2	127	41	200	92
3	226	94	296	176
4	226	140	190	126
5	64	34	60	40
6	110	67	107	69
Average	128	77	178	94
Per cent Survival	48		53	

* All figures are the average of 3 - 5 replicate plates from each of 5 random samples.

Table 9

Survival of *Escherichia coli* irradiated in buffered water and buffered water containing 5 ppm Fe as $FeCl_3$

Number of Cells x 10^4

	Non-irradiated	Irradiated		
		Control	5 ppm Fe	Reduced** light intensity
Rep. 1	162*	61	99	104
2	162	74	115	109
3	183	68	114	109
4	173	65	101	117
5	161	72	102	107
Average	168	68	107	109
Per cent Survival		40.4	64.8	63.3

* All figures are the average of 5 replicate plates of one sample.

** Light intensity reduced to the equivalent of the 5 ppm Fe treatment.

Table 10

Per cent survival of *Escherichia coli* irradiated in waters of different concentrations of a standard salt mixture*

Total Salt Concentration ppm	Per cent Survival
70 in distilled water	56
700 in distilled water	55
368 in distilled water buffered to pH 7.2	58
1088 in distilled water buffered to pH 7.2	53

* Standard salt mixture

3 parts NaCl

3 parts $CaCl_2$

3 parts $MgCl_2$

1 part KCl

Table 11

Survival of *Escherichia coli* exposed to visible light after irradiation with ultraviolet light

Number of Cells x 10^4

	Light Survivors	Dark Survivors
	94*	69
	97	75
	115	103
	42	44
Average	87	73

* Each figure is an average of 3 - 4 replicate plates from 5 random samples.

DISCUSSION

The measurements of visible light transmitted by the two clay fractions show a continual decrease in transmittance as the wavelength of light decreases. There is much less drop in transmittance of light by the 1 to 2 µ clay fraction than by the 0.1 to 0.2 µ clay fraction. Measurements of ultraviolet light transmittance demonstrates that less ultraviolet light is transmitted by the 0.1 to 0.2 µ clay fraction than by the 1 to 2 µ clay fraction.

Many of the standard turbidimeters are calibrated against suspensions made up of Fuller's earth or diatomaceous earth, and depend upon the measurement of the transmittance of visible light of wavelengths longer than 500 mµ. As evidenced by the almost permanent turbidity, many of the surface waters of the southwest contain clay particles smaller than those in the 1 to 2 µ fraction used in this work. Therefore, measurements made by the use of standard turbidimeters would not furnish adequate information on clay turbidity conditions of water to be treated with ultraviolet light. Determinations of clay turbidity by weight would be of little value unless particle sizes were known, and even this would serve only to indicate the trend expected in ultraviolet light absorption.

The biological measurements of the influence of particle size of clay suspensions on ultraviolet light

treatment of bacteria in water follow the trend of the physical measurements of light absorption. At clay concentrations of 50 ppm, survival rates of cells irradiated in the presence of clay particles from the 1 to 2 μ fraction were not significantly greater than the survival rates of cells exposed in clear water. However, the increase in survival rates of the cells exposed in 50 ppm clay suspensions of the 0.1 to 0.2 μ fraction were significantly greater than the survival rates of cells exposed to ultraviolet light in clear water. The survival rates of bacteria irradiated in suspensions of 100 and 150 ppm of the 1 to 2 μ clay fraction were significantly higher than those of the controls. The survival rates of bacteria irradiated in suspensions of 100 and 150 ppm of the 0.1 to 0.2 μ clay fraction were significantly higher than the controls and significantly higher than those of bacteria irradiated in suspensions of the same concentration of the 1 to 2 μ clay fraction. No phenomena functioning to protect the cells in turbid water from the lethal effects of ultraviolet light other than absorption of light could be demonstrated. In view of this, clay turbidity measurements based upon ultraviolet light transmittance can be used as an aid in establishing exposure time in ultraviolet treatment of water.

Bacterial cells allowed to stand for 8 to 10 hours in comparatively high concentrations of chloride salts of the

cations commonly found in water were not altered in respect to ultraviolet light resistance. It has been demonstrated that high concentrations of sodium or potassium salts in the growth medium increases the resistance of bacteria to ultraviolet light (Durham, 1954). The increased resistance may be due to the presence of the salts during the time of growth. If this be true, it is very improbable that bacteria, especially the pathogenic water-borne group, would be effected by this phenomenon after they were released into water. The absorption of light by iron in solution is apparently the only function of this ion in protecting bacteria irradiated in its presence. While the amount of iron used in this work exceeds the concentrations ordinarily found in water, it is worthy of note that the concentration of iron in water may vary over a considerable range and may influence ultraviolet treatment of water.

The survival rates of bacteria exposed to osmotic pressure differences before and during ultraviolet irradiation suggest that any difference in death rate due to the osmotic pressure differences are not additive with ultraviolet light killing effects.

Photoreactivation of small populations which were inactivated by ultraviolet light could not be demonstrated in this investigation. The informatition presented in the literature indicates that this may be

expected, since actually a small percentage of the cells available for reactivation in large populations responded to treatment with visible light. Populations of Escherichia coli as large as 2×10^9 cells per ml exposed to a dose of ultraviolet light which would produce approximately 99 per cent mortality may be reactivated to a survival rate near 10 per cent (Kelner, 1949). In smaller populations, such as those used in this work, the numbers of cells available for reactivation were approximately 5×10^5. If 10 per cent of such populations were reactivated there would be 5,000 more visible cells per ml of sample. Since a dilution of 10^4 was necessary to estimate the original population this number would be so small that detection in dilution and plating procedures would be unlikely. Unless drinking water is grossly contaminated, the expected numbers of bacteria would be less than one million per ml. The light intensities used to demonstrate photoreactivation is higher than the intensity to which irradiated bacteria in water would ordinarily be exposed. Therefore, photoreactivation is not likely to be a factor in influencing ultraviolet sterilization of water.

SUMMARY

The transmittance of visible and ultraviolet light through clay suspensions of two wide ranges of particle sizes was determined. Visible light transmittance was found to be greater through the small-particle clay fraction than through the larger-particle fraction. The reverse was found in the transmittance of ultraviolet light. Survival rates were determined for *Escherichia coli* exposed to ultraviolet light in the presence of various concentrations of each of the two clay fractions. These were demonstrated to be significantly higher for the cells exposed in the small-particle clay fraction than those exposed in the larger-particle clay fraction, when concentrations of 100 or 150 ppm were used.

Cells of *Escherichia coli* were exposed to ultraviolet light in the presence and in the absence of the cations commonly found in natural waters. The biological response of these cells to ultraviolet light was found not to be significantly different.

The survival rates of cells exposed to ultraviolet light in water at different osmotic pressures were determined. Osmotic pressure differences over the ranges studied did not influence survival.

Photoreactivation of comparatively small populations of ultraviolet irradiated cells was investigated. The

results of the experiments performed indicate that this phenomenon does not function to such a degree to be detectable under these conditions.

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APPENDIX

This appendix contains tabular data from which summary tables presented in the text were prepared.

Table 1a

Survival of <u>Escherichia coli</u> irradiated in clear and turbid water

Number of Cells x 10^4

Control

	Non-irradiated						Irradiated					
Test No.	1	2	3	4	5	6	1	2	3	4	5	6
Rep.												
1	123*	83	103	132	128	109	70	53	60	81	75	46
2	124	85	96	146	115	114	66	55	53	101	74	54
3	114	88	81	141	135	115	62	60	55	85	73	49
4	126	90	89	149	122	116	76	56	51	84	81	56
5	135	91	102	130	118	112	82	42	51	89	74	56
Mean	124	87	94	139	124	113	71	53	54	90	75	52

50 ppm 1 - 2 µ Clay

Test No.	1	2	3	4	5	6	1	2	3	4	5	6
Rep.												
1	132	98	92	143	126	83	81	63	60	76	90	54
2	131	97	91	150	115	94	95	69	58	89	88	51
3	134	125	87	146	126	91	94	71	52	93	92	60
4	128	98	89	136	124	93	73	74	55	91	86	67
5	145	95	94	137	122	94	88	62	59	86	83	68
Mean	134	103	91	142	125	91	86	68	56	87	88	60

50 ppm 0.1 - 0.2 µ Clay

Test No.	1	2	3	4	5	6	1	2	3	4	5	6
Rep.												
1	135	85	98	153	114	100	92	62	70	98	87	61
2	141	90	89	155	117	106	94	63	73	106	86	64
3	146	86	104	150	108	114	102	62	72	105	79	70
4	124	95	101	148	107	102	94	74	71	96	85	68
5	145	91	100	151	116	108	85	64	78	92	82	86
Mean	138	89	98	151	112	106	93	65	73	99	84	69

* All figures are the average of 5 replicate plates from 1 sample.

Table 2a

Survival of <u>Escherichia</u> <u>coli</u> irradiated in clear and turbid water

Number of Cells x 10^4

Control

Test No.	Non-irradiated						Irradiated					
	1	2	3	4	5	6	1	2	3	4	5	6
Rep. 1	152*	128	88	97	190	195	81	90	35	30	88	130
2	146	119	85	87	194	201	111	88	32	35	95	131
3	141	126	78	98	180	192	85	92	33	25	85	127
4	149	124	89	94	150	208	84	86	39	27	91	101
5	130	122	80	96	176	191	89	83	33	26	78	128
Mean	143	122	84	94	178	197	90	88	34	30	87	123

100 ppm 1 - 2 μ Clay

Test No.	1	2	3	4	5	6	1	2	3	4	5	6
Rep. 1	145	117	81	97	190	207	86	92	40	41	108	144
2	141	118	74	87	201	192	98	87	42	40	116	136
3	146	128	90	98	180	169	99	103	38	40	114	140
4	145	127	82	96	197	192	97	120	41	44	104	142
5	142	122	83	97	176	199	88	92	44	41	111	153
Mean	144	122	82	95	188	192	94	98	41	41	110	142

100 ppm 0.1 - 0.2 μ Clay

Test No.	1	2	3	4	5	6	1	2	3	4	5	6
Rep. 1	139	107	86	98	186	201	121	96	62	54	138	164
2	171	109	87	98	194	198	149	92	64	67	126	157
3	141	108	85	93	174	208	133	97	56	66	134	161
4	148	120	79	106	153	211	107	90	47	65	128	170
5	145	121	76	103	175	203	118	89	65	69	139	175
Mean	149	113	82	97	176	204	125	95	59	64	133	165

* All figures are the average of 5 replicate plates from 1 sample.

Table 3a

Survival of Escherichia coli irradiated in clear and turbid water

Number of Cells x 10^4

Control

	Non-irradiated				Irradiated			
Test No.	1	2	3	4	1	2	3	4
Rep.								
1	88*	198	190	172	35	132	88	109
2	86	201	194	179	32	133	89	99
3	76	192	182	181	34	119	88	107
4	89	208	148	178	39	125	79	101
5	77	192	176	190	33	122	78	95
Mean	83	198	178	180	34	126	83	102

150 ppm 1 - 2 µ Clay

Test No.	1	2	3	4	1	2	3	4
Rep.								
1	85	199	191	167	53	150	118	127
2	82	207	203	183	44	149	119	124
3	81	192	182	182	54	155	116	133
4	86	169	195	196	43	148	98	118
5	80	190	176	190	44	157	111	129
Mean	83	191	189	182	47	152	112	122

150 ppm 0.1 - 0.2 µ Clay

Test No.	1	2	3	4	1	2	3	4
Rep.								
1	86	186	202	172	62	151	175	139
2	86	194	203	175	64	142	174	154
3	85	174	198	171	56	140	168	153
4	78	151	208	179	47	142	180	143
5	76	175	215	181	64	145	186	134
Mean	82	176	205	175	58	144	176	144

* All figures are the average of 5 replicate plates from 1 sample.

Table 5a

Survival of *Escherichia coli* irradiated after standing in buffered distilled water and in 500 ppm Na as NaCl

Number of Cells x 10^4

A. 500 ppm Na

	Non-irradiated				Irradiated			
Test No.	1	2	3	4	1	2	3	4
Rep.								
1	130*	115	134	213	61	56	96	114
2	133	113	182	172	54	51	92	114
3	113	112	154	201	48	62	98	109
4	125	82	143	186	52	66	89	124
5	138	115	141	200	56	63	86	109
Average	125.8	107.4	150.8	194.4	54.2	59.6	92.2	114.0

Per cent survival 55.3

B. Buffered distilled water

	Non-irradiated				Irradiated			
Test No.	1	2	3	4	1	2	3	4
Rep.								
1	129	121	153	195	55	48	96	112
2	102	104	150	201	62	54	76	123
3	112	115	152	196	46	40	94	116
4	119	111	136	197	42	53	63	114
5	140	106	155	165	50	56	70	101
Average	120.4	111.4	149.2	190.8	51	50.2	77.8	113.2

Per cent survival 50.7

* Each figure represents the average of 3 replicate plates.

Table 6a

Survival of *Escherichia coli* irradiated after standing in buffered distilled water and 50 ppm K as KCl

Number of Cells x 10^4

A. 50 ppm K

	Non-irradiated		Irradiated	
Test No.	1	2	1	2
Rep. 1	172*	141	64	58
2	169	155	59	75
3	145	179	61	82
4	152	223	45	76
5	134	159	54	46
Average	154.2	171.2	56.6	67.4

Per cent survival 38.0

B. Buffered distilled water

	Non-irradiated		Irradiated	
Test No.	1	2	1	2
Rep. 1	126	213	32	86
2	124	221	43	91
3	136	195	36	96
4	104	214	41	92
5	116	214	33	89
Average	121.2	211.4	37.0	90.8

Per cent survival 38.4

* Each figure represents an average of 3 replicate plates.

Table 7a

Survival of *Escherichia coli* irradiated after standing in buffered distilled water and in 500 ppm Ca as $CaCl_2$

Number of Cells x 10^4

A. Buffered distilled water

	Non-irradiated		Irradiated	
Test No.	1	2	1	2
Rep. 1	117*	216	52	86
2	133	205	55	88
3	147	239	51	88
4	111	224	48	87
5	113	213	59	86
Average	124.2	219	53	87

Per cent survival 40.7

B. 500 ppm Ca

	Non-irradiated		Irradiated	
Test No.	1	2	1	2
Rep. 1	131	226	58	116
2	123	260	50	133
3	117	216	55	106
4	112	244	56	108
5	109	270	51	108
Average	118.4	243.2	54	114.2

Per cent survival 46.5

* Each figure represents an average of 3 replicate plates.

Table 8a

Survival of _Escherichia coli_ irradiated after standing in buffered-distilled water and in 500 ppm Mg as $MgCl_2$

Number of Cells x 10^4

A. Buffered-distilled water

Test No.	Non-irradiated 1	2	3	4	5	6	Irradiated 1	2	3	4	5	6
Rep. 1	204	144	235	208	63	104	82	43	103	132	36	68
2	214	114	214	214	55	92	95	40	98	145	33	70
3	216	121	226	230	68	121	96	43	82	140	32	69
4	225	129	241	235	70	111	81	40	90	147	36	62
5	221	138	214	244	66	123	77	39	99	138	34	70
Average	216	127.4	226	226.2	64.4	110.2	86.2	41.0	94.4	140.4	34.2	67.8

Per cent survival 47.8

B. 500 ppm Mg

Test No.	Non-irradiated 1	2	3	4	5	6	Irradiated 1	2	3	4	5	6
Rep. 1	237	221	287	170	67	104	68	85	172	118	43	63
2	230	204	293	207	57	111	58	76	177	138	38	69
3	213	193	286	203	66	106	63	111	178	125	36	72
4	235	200	301	179	60	108	62	89	176	125	42	72
5	117	185	316	193	52	109	67	89	181	126	34	71
Average	217.2	200.6	298.6	190.4	60.4	107.6	63.6	92.0	176.8	126.4	38.6	69.4

Per cent survival 52.8

Table 10a

Survival of *Escherichia coli* irradiated in different concentrations of a standard salt mixture

Number of Cells x 10^4

Salt Concentration ppm		Non-Irradiated	Irradiated	Per cent Survival
		124*	71	
		131	68	
70		126	70	56.4
		117	71	
		126	72	
	Means	124.8	70.4	
		112	66	
		113	64	
360		102	66	57.6
		115	60	
		110	62	
	Means	110.4	63.6	
		105	61	
		112	58	
700		117	65	55.4
		124	68	
		112	64	
	Means	114	63	
		105	49	
		103	61	
1080		114	64	52.9
		106	52	
		106	57	
	Means	106.8	56.6	

* Each figure is an average of 3 - 4 plates.

Table 11a

Survival of *Escherichia coli* exposed to visible light after irradiation with ultraviolet light

Number of Cells x 10^4

	Light Survivors				Dark Survivors			
Test No.	1	2	3	4	1	2	3	4
Rep.								
1	93*	89	113	36	78	78	88	33
2	98	100	117	47	60	74	102	45
3	88	101	112	43	67	70	107	50
4	103	101	120	41	79	80	110	47
5	88	94	116	42	65	71	111	46
Average	94	97	115	42	69	75	103	44

* Each figure is an average of 3 - 4 replicate plates.

VITA

Paul Edward Degurse
Candidate for the degree of
Master of Science

Thesis: THE INFLUENCE OF VARIOUS FACTORS ON THE ULTRAVIOLET LIGHT STERILIZATION OF WATER

Major: Bacteriology

Biographical and Other Items:

Born: January 29, 1920 at Trinidad, Colorado.

Undergraduate Study: Trinidad State Jr. College, 1940-1941; The University of Colorado, 1945-1949.

Graduate Study: The University of Colorado, 1949-1950; O. A. M. C., 1953-1955.

Experiences: U. S. Army. Served in England, Africa, Sicily and Italy, 1941-1945; Laboratory Assistant, Biology Department, University of Colorado, 1948-1950; Instructor in Bacteriology, Summer, 1950, University of Colorado; Bacteriologist, Wisconsin Conservation Department, 1950-1953; Graduate Assistant, Bacteriology Department, O. A. M. C., 1953-1955.

Member of The Society of American Bacteriologists and Phi Sigma.

Date of Final Examination: May, 1955.

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AUTHOR: Paul Edward Degurse

THESIS ADVISER: Dr. Lynn L. Gee

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