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The effect of ultra-violet and infra-red rays upon the fission rate within a single clone of paramecium caudatum and the heritability of that effect.

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THE EFFECT OF ULTRA-VIOLET AND INFRA-RED RAYS UPON THE FISSION RATE WITHIN A SINGLE CLONE OF PARAMECIUM CAUDATUM AND THE HERITABILITY OF THAT EFFECT

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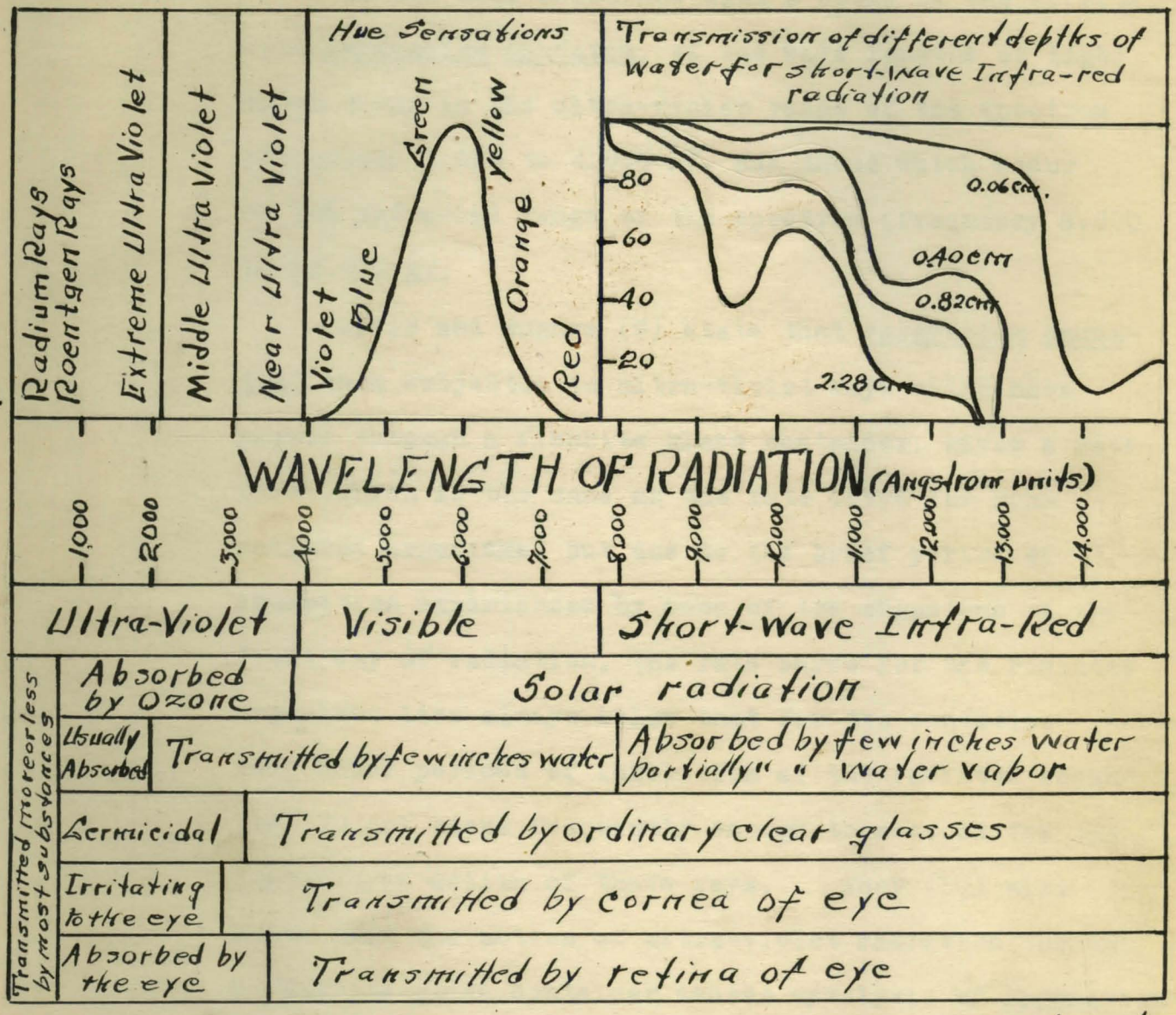
My deep gratitude is due Dr. Austin R. Middleton, Director of the Biological Laboratories of the University of Louisville, for his gracious guidance and constructive criticism of this work. Dr. Middleton has given unstintingly of the results of his numerous researches done within the clone of *Paramecium caudatum* and this work is hereby dedicated to him.

INTRODUCTION

Light in its various phases exerts a marked influence on the functional as well as the physical structure of all living matter. The phenomena of light involves two theories: (1) the wave or electro-magnetic theory conceiving light as consisting of electric emanations or waves of various frequencies and intensities given off from many substances. (The principal source of light as thus conceived is the sun with its estimated temperature of 10,000 F.°); (2) the quantum theory which conceives light as a factor involved in ionization in chemical reactions [I] (negative electrons are ejected from a surface of metallic iron when a strong light is focused upon it; i. e., the light causes a quantum of ionization in the iron.) Figure 1 is a diagram showing some of the important physical properties of light.

[I] Quantum - a unit of light energy equal to (hn) where (n) is the frequency of passage of the negative ion around its positive nucleus, and (h) is a unit of action or quantum. Planck's constant = (h) = 6.55 10^{-27} erg-seconds.



— M. Luckiesh

10.000 Å = 1.000 μμ = μ = .001 = .0001 cm.

Fig. 1. Diagram showing the various properties of light.

We are here concerned with a study of the effects, upon Paramecium caudatum, of the wave lengths of light which occur in the ultra-violet range of the spectrum (frequency 1.000 to 4.000 Å), and those which occur in the infra-red range of the spectrum (frequency 8.000 to 15.000 Å).

Bovie and Hughes (9) state that Paramecium caudatum, when subjected to ultra-violet rays which heve passed through a fluorite glass container, gives a rate curve which is the same as the rate curve for non-radiated organisms, but due to the brief period of inhibition experienced by some of the organisms on the first day of radiation, the rate curve for the radiated organisms lies always below that for the controls. Very brief periods of inhibition of the radiated organisms do not occur frequently enough to account for the destructive action of these rays. They (10) also state that the action of ultra-violet radiation through a fluorite glass container causes cytolysis of Paramecium caudatum when a certain amount of a toxic "photo-product" is formed. In their experiments this occured in 57 per cent of the organisms, as a result of two exposures of four seconds each, separated by a two second interval. Recovery depends upon the ability of the organism to

eliminate this toxin through a combination of chemical action and diffusion.

Crosley [3] states that it has been found that short time-duration-exposures, (exposures at specific distances from source of radiation for specific time intervals) of ultra-violet radiation stimulate animal tissues, while time-distance-exposures of longer duration result in a slowing down of physiological activity of tissues. Time-distance-exposures of extremely long duration results in the destruction of the animal tissues. In the plant kingdom it has been found that ultra-violet radiation of certain time-distance-exposures results in an increase in growth and cane-sugar content of sugar cane. Crosley also states that periods of exposure of Paramecium caudatum to ultra-violet radiation necessary for death have been prolonged by simultaneous exposure to infra-red rays.

For single-celled organisms short time-distance-exposure to light of a wave length of 280 μμ (2800 Å) results in a maintenance of motility. Longer time

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[3] Personal letter from G. E. Crosley of Burdick Corperation, Milton, Wisconsin.

exposures are lethal. Light radiations of 160 (1600 Å) are absorbed by the cytoplasm of the cell, while radiations of wave length 185 μμ (1850 Å) are absorbed by the nucleus of the cell. These radiations absorbed by the cell nucleus interfere with cell metabolism and finally cause death. Radiations of 185 of short time-distance-exposure temporarily inhibit cell division, while radiations of 185 of long time-distance-exposure stimulate cell division. Those radiations absorbed by the cytoplasm (160 μμ) in short time-distance-exposure stimulate aimless motion of infusoria or incite phagocytosis by them, while longer time-distance-exposures result in necrosis of the cell with accompanying vacuolization of the irradiated cell cytoplasm. Radiations of 160 μμ as transmitted by fluorite glass, are practically all absorbed by or in the cell cytoplasm of Amoeba proteus. The radiation causes coagulation and excessive vacuolization of the cytoplasm. The effected part of the amoeba becomes immobilized and the uninjured portion separates from the rest of the mass.

Ultra-violet radiations of wave length 185 μμ passing through quartz containers are only slightly absorbed by cell cytoplasm and strongly absorbed by

the cell nucleus. Prolonged exposure to radiations of this wave length may be followed by inhibition of cell division without any visible effect upon motility. Inhibition of cell division, following short time-distance-exposures to "quartz rays", is followed by a period of accelerated cell division so that after a given period the irradiated organisms reproduce more rapidly than do the controls. This action of short time-distance-exposures to "quartz rays" may be due to a toxic "photo-product" consisting probably of lecithins, which in minute residual excreted quantities may act as a stimulus to cell division. One-thirtieth of lethal exposure (of a concentration necessary to cause cytolysis) inhibits the organism. It becomes thin and transparent, and in appearance resembles the enucleated and immobilized fragment of Amoeba as described above, but in Paramecium caudatum this change is only temporary.

Ultra-violet radiation acts directly on the chromatin (nucleo protein) of the exposed cell which may liberate some stimulative toxin. There is also the possibility that the increased activity may be due to the stimulative or the destructive action of

perioxidase or invertase; or the increased output of energy may be due to the absorption of ultra-violet rays by the amino acids of the cell.

Forbes and Daland (26) found that there is a sensitization to heat caused by exposure of Paramecium caudatum to ultra-violet rays passing through a fluorite container. Death results either from the direct action of the rays or from the ozone formed in the reaction. Heat following strong radiation has apparently a more lethal effect upon the organism than heat preceding it.

Bovie (6) states that when Paramecium caudatum is exposed to sub-lethal time-distance-exposure of ultra-violet rays, the organism is killed by an amount of heat which would not effect a normal unradiated paramecium. Uniqueness of the effect of ultra-violet radiation lies in the fact that these rays form within the cell the chemical combinations which are foreign to normal protoplasm. The radiated organism which has not been exposed to an increase in temperature may appear quite as normal as if the rays had no effect whatsoever upon it. It is only when the "photo-chemical change" or "latent image" has been developed by the metabolic changes occuring at higher temperatures that

the effects of the exposure are to be observed.

Packard (47) finds that there is a susceptibility of living cells to radium radiations of wave lengths below 800 Å in the extreme ultra-violet range of the spectrum. This susceptibility of Paramecium to radium radiations chiefly of the slowest β rays (electrons moving with very high velocities) varies with the temperature at the same rate as do the physiological reactions of various kinds. It also varies directly with the degree of permeability of the surface layer of the cell. The slow rays act on the surface layer of the cell increasing its permeability and if allowed to act long enough cause a typical cytolysis. In this respect rays resemble other types of radiant energy and diverse chemical cytolytic agents. Cells which have a relatively high permeability are more susceptible to the lethal action of rays of radium than those having a low permeability; for the cytolytic action of these rays is quickly followed in the former by a cytolysis which is irreversible, while in the latter case the reaction is a reversible one.

Soret (28) pointed out that the majority of

proteins show a well marked absorption band in the ultra-violet range of the spectrum. He attributed this phenomena to the presence of the "tyrosine radicle" in the protein molecule.

More recent investigation by Koher (36) of the spectrographic action of chemical solutions of the various amino acids has corroborated the work of Soret and enlarged upon it. Among the constituents of the protein molecule, phenylalanine, as well as tyrosine, exhibits a well marked absorption band in the ultra-violet range of the spectrum. Other amino acids, constituents of the protein molecule, exhibit only general non-selective absorption bands in the ultra-violet range of the spectrum. The tyrosine and phenylalanine radicles of protein molecules constitute the optical sensitizers which render living cells receptive to the toxic action of ultra-violet rays.

The above assertions are further substantiated by the following experiment conducted by Harris and Hoyt (28). A double container, the bottom section composed of silicon glass and the top section of quartz, was used. Paramecia were placed in the bottom section

and various amino acids in solution were alternately placed in the top section of the double container. The container was then exposed to the rays of a mercuric arc lamp in a quartz container in such a way that the rays passed through the solutions of the amino acids before they reached the paramecia in the bottom section of the container. Amino-benzoic acid had the strongest power of absorbing the toxic rays followed in order by tyrosine, leucine, glutamic acid, aspartic acid, and alanine.

Tappeiner, Osthelder, and Erhart (57) state that various chemicals cause photodynamic changes which occur in protoplasm when it is exposed to ultra-violet light. When paramecia are treated in the dark with eosin, sodium dichloranthracenedisulphonate, and methylene blue hydrocloride and then brought into the ultra-violet light, the ultra-violet rays cause all the above dyes to be taken up peripherally.

Hausmann and Kalmer (29) find that both plant and animal coloring matters have a sensitizing action on paramecium. Basic dyes of this sort, such as small amounts of methol orange, do not act peripherally on paramecium, but their counterparts do act peripherally

on paramecium. The neutral coloring matters that are active upon erythrocytes are also active upon paramecium in light.

Metzner (43) states that paramecia can be made sensitive to light by the addition to their suspensions of fluorescent coloring materials such as erythrosin and eosin. This photo-taxic activity, whether positive or negative, can be induced as long as the organism is not harmed by a too strong photodynamic action. The strength of this photodynamic action is proportional to the light and color intensity, and the oxygen concentration. In favorable cases the light effects follow radiation within 0.1 second. The maximum effect is induced by the maximum non-lethal amount of coloring matter dissolved in or combined with the living protoplasm of the animal cell. Oxidase and catalase play important roles in the causation of the photo-taxic reactions.

Ball (2) finds that the only dyes which stain the cytoplasm of normal living paramecia belong to the basic group. The most suitable are: Bismarck brown, methylene blue, methylene green, neutral red, and toluidine. The cytoplasm of the normal organisms cannot be stained by any

of the acid dyes used, although these might stain the contents of the food vacuoles of the dead or dying organisms. The exposure to light of paramecia having the cytoplasm stained by certain dyes, or of paramecia in a concentrated solution of eosin, produces a marked avoiding reaction within five seconds after exposure. The organisms in this reaction die more rapidly in strong light.

Tappeiner (57) arrived at the following conclusions regarding the phenomena of light as related to the photodynamic reactions in living matter.

(1). The photodynamic substances act only in the wave lengths which the fluorescent substance absorbs;

(2). Photodynamic substances act on enzymes;

(3). Only substances which fluoresce in water solutions are active as sensitizers;

(4). In some cases photodynamic substances act on the outside of the organism, and in some cases these substances act within the organism;

(5). The substance used must fluoresce, but the amount of action does not vary with the amount of fluorescence;

(6). The fluorescent radiations are not effective nor is there a photo-electric effect evident;

(7). The fluorescent substance must come into contact with the organism. Action therefore seems to be due to some activity of the electrons; and

(8). The action of the fluorescent substance does not depend on the presence of free oxygen.

Von Baeyer's conception of the origin of life (41) was that carbon dioxide and moisture (water), under the influence of sunlight, formed formaldehyde, and this in turn acted upon by sunlight produced starches, sugars, and the complex organic compounds found in plants.

Moore and Webster (41), a few years ago, succeeded in building up organic compounds from inorganic systems by the use of ultra-violet light. The method employed, in brief, consisted in exposing colloidal hydrated ferric oxide (0.113% Fe_2O_3) or colloidal oxide of uranium (0.028% uranium) in a quartz container, through which carbon dioxide was bubbled, to the radiations of a mercuric arc lamp walled in quartz. The reactions obtained in this experiment were similar to those occuring in the first stage of the synthesis of organic from inorganic substances as it occurs in green plants through the agency of chlorophyll.

THE SPECIFIC PROBLEM

When a single infusorian divides, often one of its two progeny divides before the other does. In successive generations this same thing may be repeated. Thus, as shown in figure 2, one may have among the progeny of a single individual at a given moment, animals that are the products of two and others that are the products of three fissions. Under these circumstances selection for either fast or slow rate of fission is inevitable. Middleton (45), by his devise of 'balanced selection', eliminated this selection. This opened the possibility of accurately testing the effects of environmental factors upon the hereditary fission rate. The first of these studies was that of Middleton (45) in which it was shown that temperature differences produce heritable differences in the fission rate. Subsequent unpublished research of Professor Middleton has shown that both organic and inorganic chemical compounds also modify the fission rate in the hereditary sense. At the suggestion of Professor Middleton, I have undertaken to test the effects of ultra-violet and infra-red radiations upon the hereditary fission rate of the Protozoa.

No previous investigation has been made of this

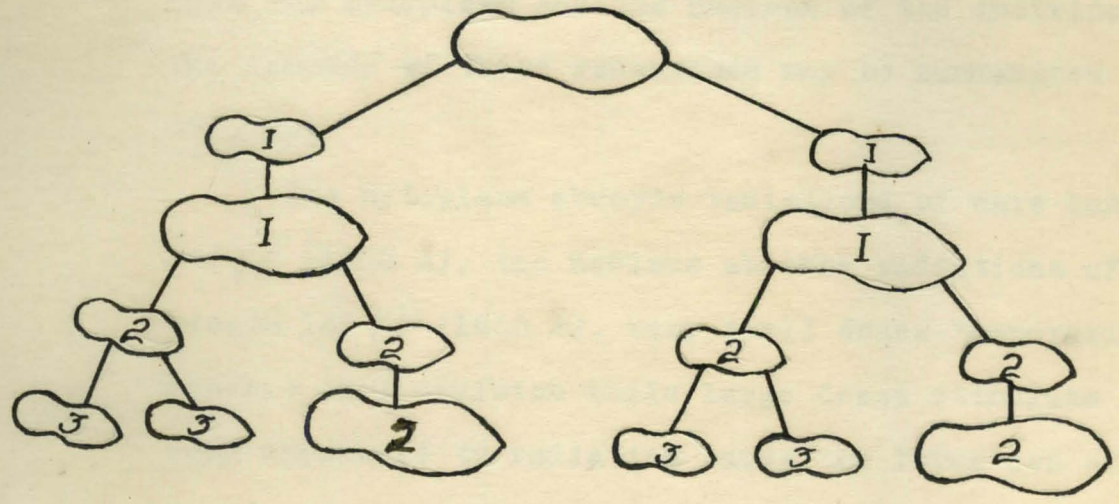

Fig. 2. Diagram of successive fissions among the progeny of a single parent, illustrating the variations in fission rate which occur and which were subjected to balanced selection in the present experiments.

important question. Previous studies of the effects of these radiations have been concerned with the investigation of the physiological effects of these radiations upon the cytoplasm and the nucleus of the individual cell. The results of these researches may be summarized as follows.

The cytoplasm absorbs radiations of wave length 160 μμ (1600 Å), the nucleus absorbs radiations of wave length 185 μμ (1850 Å), very small doses temporarily inhibit cell division while large doses stimulate it. Long exposures to radiation cause the formation of a toxic substance within the cell which is lethal in its effect. The rate of recovery of the cell from sub-lethal doses of radiation depends upon the removal of the toxic substance through a process of chemical action and diffusion. Paramecium can be made sensitive to light by the addition of fluorescent coloring matters such as erythrosin and eosin to the culture medium. Heat following strong radiation has apparently a more lethal effect than heat preceding it. Paramecium, when exposed to a sub-lethal dose of radiation, is killed by an amount of heat which would not effect a normal unradiated animal.

Only dyes belonging to the basic group stain the cytoplasm of normal living paramecia. On exposure of the animal to light these dyes are rapidly voided. Under the influence of light paramecium takes up eosin, sodium dichloranthracenedisulphonate, and methylene blue hydrochloride peripherally. Neutral coloring matter of both plant and animal derivation is active upon paramecium in the presence of light.

The protoplasm of paramecium contains various nitrogenous products, among which are tyrosine, phenyl-alinine, and amino benzoic acid, which show well marked absorption bands in the ultra-violet range of the spectrum. The nucleo proteins thymine, cystine, and uracil also show absorption bands in the ultra-violet range of the spectrum.

Paramecium caudatum when exposed intermittently to infra-red radiations is made more resistant to the lethal action of large doses of ultra-violet radiations. To date no further significant research relative to the effects of infra-red radiation upon Paramecium caudatum has come to my notice.

Are the effects upon Paramecium caudatum , induced by ultra-violet and infra-red radiations merely transient

Or are they heritable?

The present paper is a statement of the results of a series of experiments designed to throw light upon the question of the possibility of modifying living systems by environmental factors so that the modifications persist in later generations in the absence of the factor which called them forth. Can we change the living system, by subjecting it to invisible rays, so that the changes persist through later generations?

MATERIALS AND METHODS

In order that results obtained in experimentation designed to test the heritability of the effect of a single environmental factor upon an hereditary character in organisms, it is essential that the environments of the two sets of organisms, experimental and control, shall be absolutely identical save in respect to the single factor that is under investigation. This is particularly true when the hereditary character concerned is a physiological character which is so delicately responsive to all environmental changes as is the fission rate of infusoria, the organisms used in these experiments.

Jennings (32) has pointed out that to secure this uniformity the bacterial content must not vary. Further, the technique used in work on the fission rate must insure the experimenter against admixture of the various pure lines of the clone used, as well as against the chance introduction of a 'wild' individual into any of the lines. The culture medium used must also be uniformly unvarying in character. These results have been secured by the adoption of a modification of the method described by

Jennings (32).

The culture medium used was a $\frac{1}{16}$ of one per cent solution of Horlick's malted milk. A fresh supply was made daily. This is the medium adopted by Miss Peebles (49). One gram of the malted milk powder was dissolved in a 100 cc graduate in a few cc. of boiling glass-distilled water; this was then diluted to 100 cc. with more of the boiling glass-distilled water. Six and one-quarter cc. of this one per cent solution were then diluted to 100 cc. with boiling glass-distilled water and this $\frac{1}{16}$ per cent solution was filtered and cooled. After each such preparation of the culture medium the various vessels used were thoroughly washed in boiling glass-distilled water. The above procedure insures the experimenter against any variation whatever in the chemical composition of the medium.

The animals were cultivated on ground glass slides having each two circular depressions capable of holding four or five drops of liquid each. These were kept in moist chambers. Three drops of culture medium were used in each depression and no cover glasses were employed. The twenty ultra-violet lines were kept in one moist

chamber, and the twenty infra-red lines were kept in another moist chamber, and the twenty control lines in a third moist chamber.

Uniformity of bacterial content was secured by washing each animal in fresh culture medium before transferring it to a new slide. The animals were allowed to swim about for a time in the fresh medium, in order to wash themselves largely free from bacteria; they were then transferred to the definitive slide in other fresh medium. There was a daily cross-inoculation of the culture medium in the various concavities. The pipette and wash-slide used in transferring the individuals were invariably sterilized in boiling glass-distilled water after each transfer, thus absolutely preventing the unintentional introduction of any individual which might cling to the pipette; there was thus no possibility of admixture or contamination of lines. The slides were labeled in lead pencil; the number of fissions and selections at each examination were likewise recorded on them, to be later transferred to permanent records. The individual lines were numbered consecutively from one to twenty inthe experimental and the control sets. The slides, beakers, graduates,

and pipette were daily washed in boiling glass-distilled water.

Each concavity contained characteristically one individual. This is essential in the determination of the true fission rate. When two animals are used at each transfer it is not possible to be certain as to the number of fissions represented by certain of the animals which may be present. This is demonstrated by figure 3 which shows the possibility of the simultaneous presence in a groove of animals which are the result of one fission, two fissions, and three fissions respectively; without the possibility of differentiating those which are the result of two fissions from those which are the result of three fissions. The lines were continued under balanced selection which was devised by Middleton (45).

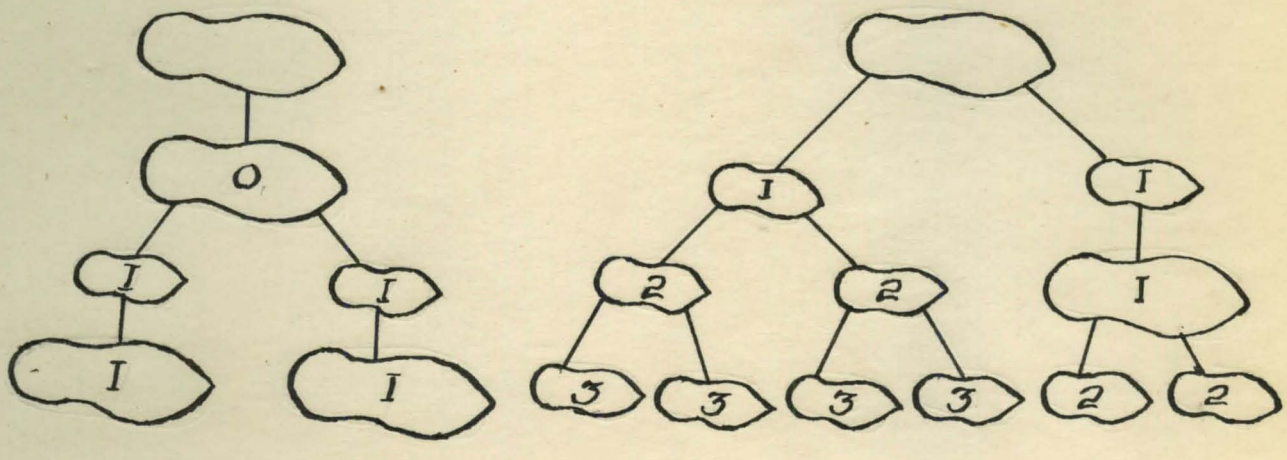

Fig. 3. Diagram of successive fissions among the progeny of two individuals, showing that at a given moment we may have, among the progeny of these two parents, individuals of the second and the third generations which are indistinguishable. The numbers within the outlines of the animals indicate the number of fissions which have intervened between it and its original parent.

EXPERIMENTS AND DISCUSSIONS

Experiments To Test The Effect Of Ultra-Violet And Infra-Red Rays Upon The Fission Rate Within A Single Clone Of Paramecium Caudatum And The Heritability Of That Effect.

Experiment 1. The effect of ultra-violet light, October 12 to October 26, 1928. On October 12, twenty selected animals of fast fission rate of eighty days balanced selection, were exposed to ultra-violet rays of wave length 1849 Å to 3900 Å, at a distance of thirty inches from a Burdick-Cooper-Hewitt mercury anode tungsten cathode quartz lamp. The animals were exposed for thirty seconds each day of a fifteen day experimental period. Twenty selected animals of this same set of lines were kept under balanced selection as controls. Figure 4 is a graph of variation in actual number of fissions of the radiated set of animals, and of its control set of non-radiated animals for the fifteen day experimental period. The radiated animals averaged 8.35±.07 fissions per line for the fifteen day period, with a range of from 4 to 11 fissions per line; in the same period the control set of non-radiated animals averaged 23.±.06 fissions per line, with a range of from 20 to 27 fissions per line.

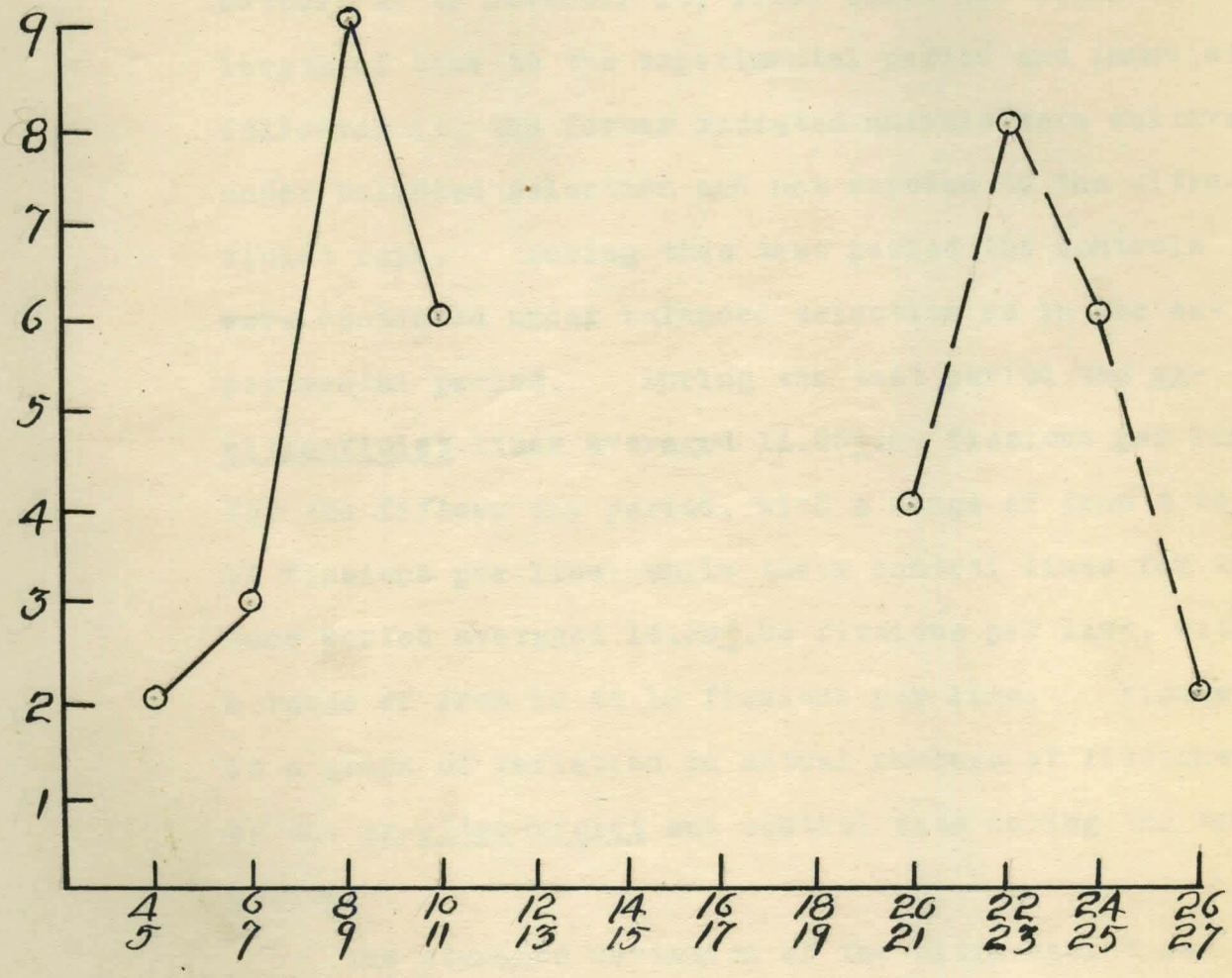

Fig. 4. Graph of variation in actual number of fissions of the radiated set of animals and of its control set of non-radiated animals for the fifteen day experimental period. Ordinates represent occurence of lines; abscissae, the number animals per line.

For a test period of fifteen days duration, from October 27 to November 10, 1928, which was equal in length of time to the experimental period and immediately following it, the former radiated animals were cultivated under balanced selection and not exposed to the ultra-violet rays. During this test period the controls were continued under balanced selection as in the experimental period. During the test period the ex-ultra-violet lines averaged 11.05±.09 fissions per line for the fifteen day period, with a range of from 4 to 14 fissions per line; while their control lines for the same period averaged 14.28±.03 fissions per line, with a range of from 10 to 18 fissions per line. Figure 5 is a graph of variation in actual numbers of fissions of the ex-ultra-violet and control sets during the test period.

The standard deviation of the ultra-violet set was 1.96_.03 and its control set was 1.80±.03; while the standard deviation for the ex-ultra-violet set was 2.73±.04 and its control set was 1.92±.03.

The coefficient of variability for the ultra-violet set was 21.00 and its control set was 7.82; while the coefficient of variability for the ex-ultra-violet set

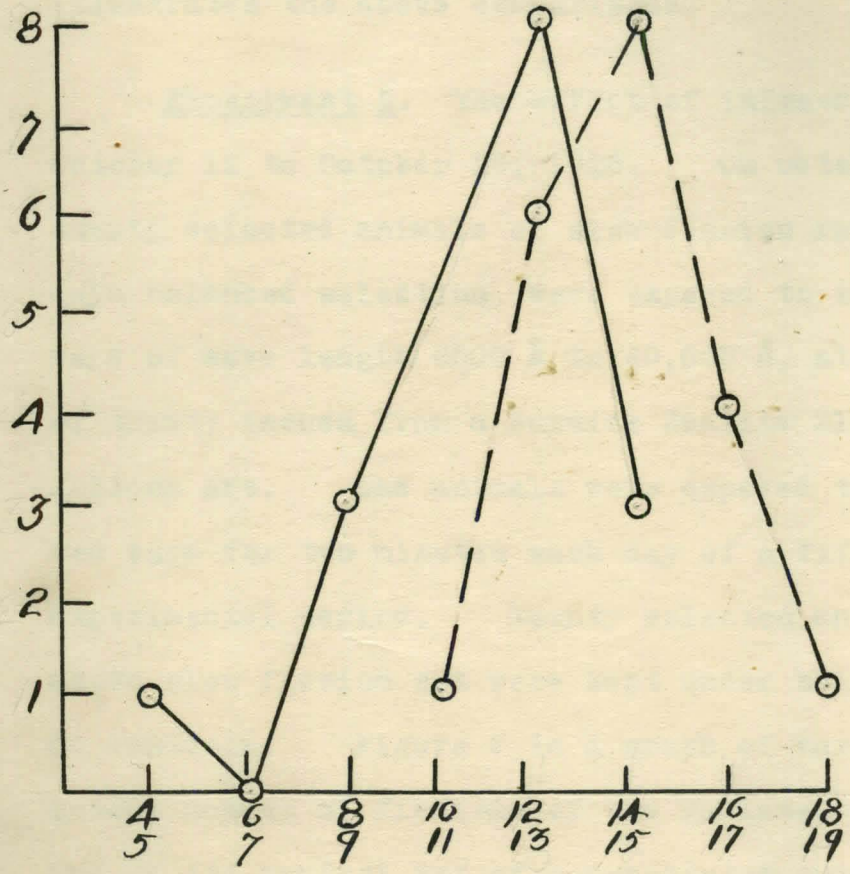

Fig. 5. Graph of variation in actual number of fissions of the ex-ultra-violet and control sets during the fifteen day test period. Ordinates represent occurence of lines; abscissae the number of animals per line.

was 24.70 and its control set was 13.40. Table 3 illustrates the above comparisons.

Experiment 2. The effect of infra-red light, October 12 to October 26, 1928. On October 12, twenty selected animals of slow fission rate of eighty days balanced selection, were exposed to infra-red rays of wave length 6500 Å to 40,000 Å, at a distance of thirty inches from a Burdick Zoalite Z12 carbo-silicon arc. The animals were exposed to the infra-red rays for two minutes each day of a fifteen day experimental period. Twenty selected animals of the above slow fission set were kept under balanced selection as controls. Figure 6 is a graph of variation in actual number of fissions of the radiated set of animals, and of its control set of non-radiated animals for the fifteen day experimental period. The radiated animals averaged 22.75±.06 fissions per line for the fifteen day period with a range of from 19 to 26 fissions per line; in the same period the control set of non-radiated animals averaged 19.40±.04 fissions per line for the fifteen day period, with a range of from 14 to 23 fissions per line.

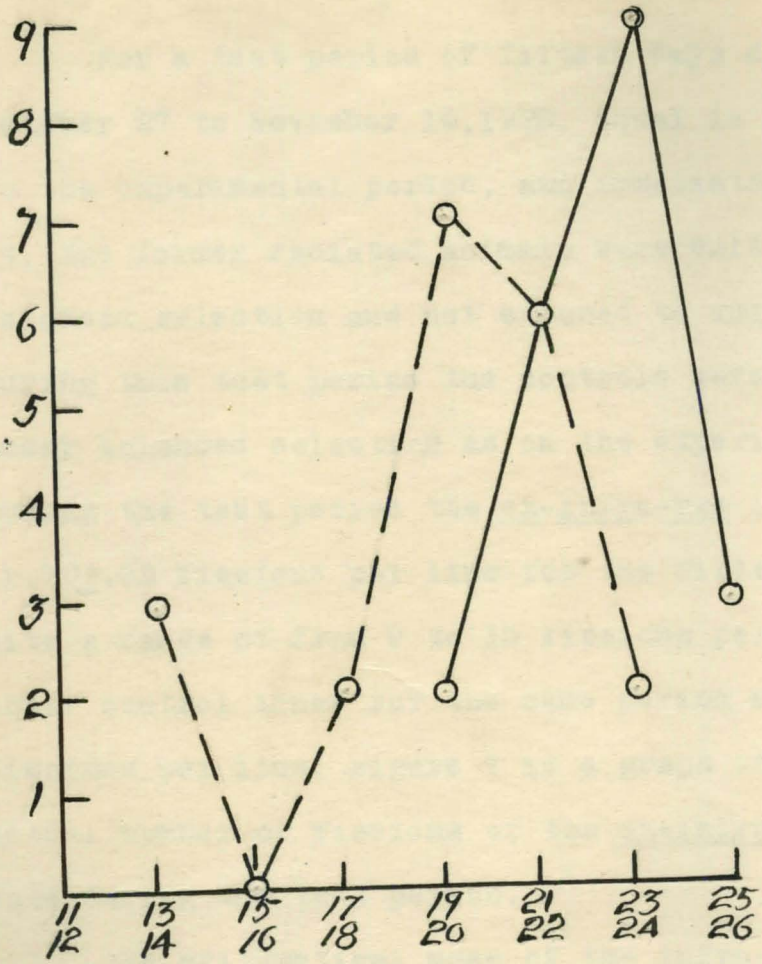

Fig. 6. Graph of variation in actual number of fissions of the radiated set of animals, and of its control set of non-radiated animals for the fifteen day experimental period. Ordinates represent occurence of lines; abscissae the number of animals per line.

For a test period of fifteen days duration, October 27 to November 10,1928, equal in length of time to the experimental period, and immediately following it, the former radiated animals were cultivated under balanced selection and not exposed to infra-red rays. During this test period the controls were continued under balanced selection as in the experimental period. During the test period the ex-infra-red lines averaged 11.70±.02 fissions per line for the fifteen day period, with a range of from 9 to 15 fissions per line; while their control lines for the same period averaged 12.20±.09 fissions per line. Figure 7 is a graph of variation in actual number of fissions of the ex-infra-red and control sets during the test period.

The arithmetical mean of the infra-red radiated set is greater by 3.35 fissions per line than the same mean for its control set of non-radiated animals for the same period. The arithmetical mean of the ex-infra-red set is less by .60 fissions per line than the same mean for its control set during the same period. The above findings are significant of the transient effect of infra-red radiation upon the fission rate of

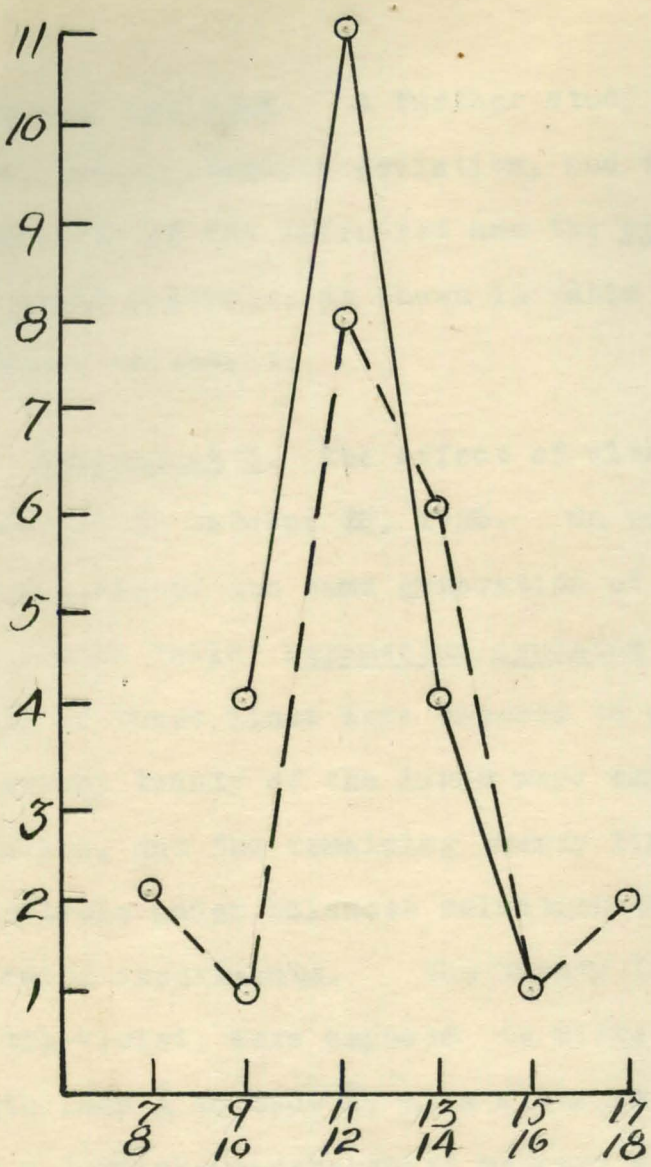

Fig. 7. Graph of variation in actual numbers of fissions of the ex-infra-red and control sets during the fifteen day test period. Ordinates represent occurence of lines; abscissae the number of animals per line.

Paramecium caudatum. A further study of the arithmetical mean, standard deviation, and the coefficient of variability of the infra-red and the ex-infra-red sets, with their controls, as shown in Table 3, corroborates the above statement.

Experiment 3. The effect of ultra-violet light, October 10 to October 25, 1928. On October 10, sixty animals, all of the same generation of the descen dants of a single "wild" Paramecium caudatum, were isolated. Twenty of these lines were exposed to ultra-violet rediation; twenty of the lines were exposed to infra-red radiation, and the remaining twenty lines were cultivated as controls under balanced selection throughout the following experiments. The twenty lines, of experiment 3 ultra-violet, were exposed to ultra-violet rays of wave length 1849 Å to 3900 Å, at a distance of thirty inches from a Burdick-Cooper-Hewitt mercury cathode tungsten anode quartz lamp. The animals were exposed to radiation for thirty seconds each day of a fifteen day experimental period. Twenty animals, selected as were the above experimental animals, were kept as controls under balanced selection.

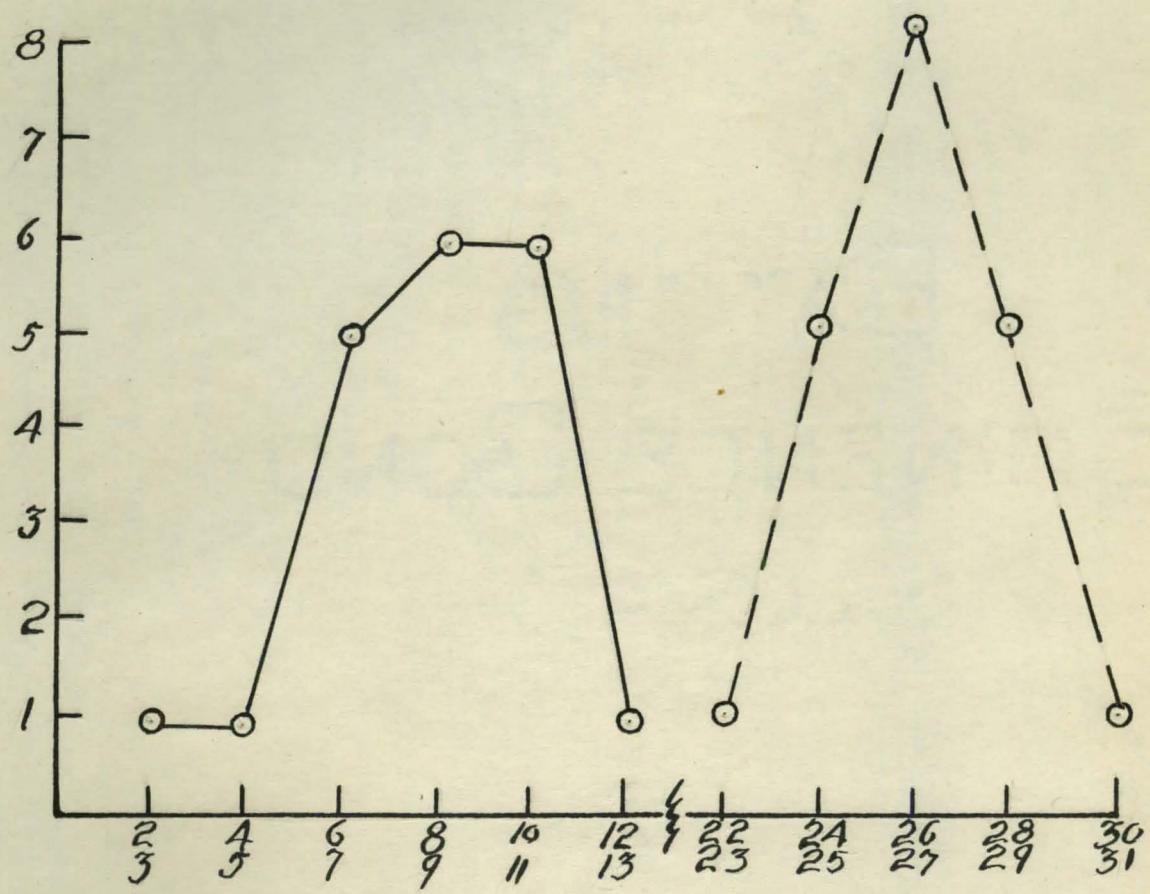

Fig. 8. Graph of variation in actual number of fissions of the radiated set of animals, and of its control set of non-radiated animals for the fifteen day experimental period. Ordinates represent occurence of lines; abscissae the number of animals per line.

Fig. 8 shows the

variation in actual number of fissions of the radiated set of animals and of its control set of non-radiated animals for the fifteen day experimental period. The radiated animals averaged 8.15±.08 fissions per line for the fifteen day period with a range of from 3 to 12 fissions per line; in the same period the control set of non-radiated animals averaged 26.65±.06 fissions per line, with a range of from 23 to 31 fissions per line.

For a test period of fifteen days duration, October 26 to November 9, 1928, equal in length of time to the experimental period and immediately following it, the former radiated animals were cultivated under balanced selection and not exposed to ultra-violet rays. During this period the controls were continued under balanced selection as in the experimental period. During the test period the ex-ultra-violet lines averaged 6.10±.04 fissions per line for the fifteen day period, with a range of from 0 to 10 fissions per line; while their control lines for the same period averaged 13.30±.08 fissions per line, with a range of from 10 to 20 fissions per line. Figure 9 is a graph of vatiation in actual number of fissions of the ex-ultra-violet and the control sets during the test period.

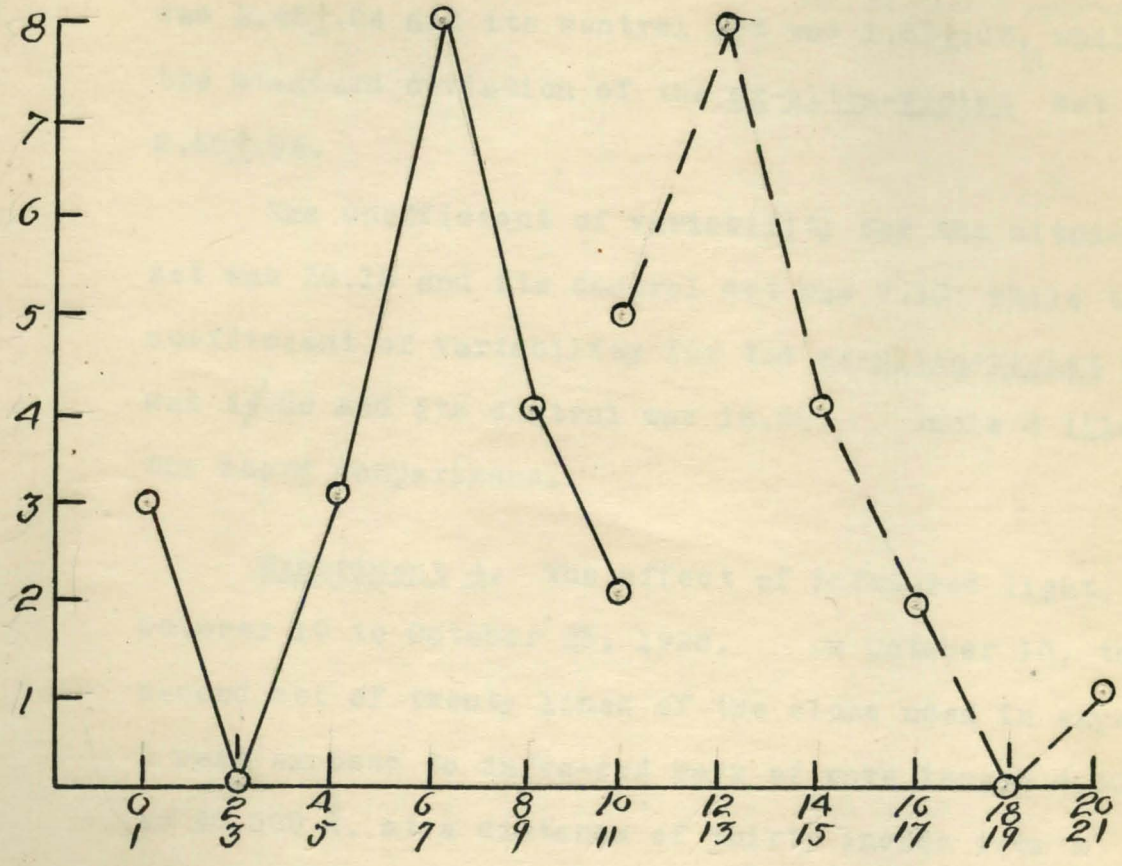

Fig. 9. Graph of variation in actual number of fissions of the ex-ultra-violet and control sets during the fifteen day test period. Ordinates represent occurence of lines; abscissae the number of animals per line.

The standard deviation of the ultra-violet set was 2.46±.04 and its control set was 1.89±.03, while the standard deviation of the ex-ultra-violet set was 2.50±.04.

The coefficient of variability for the ultra-violet set was 30.18 and its control set was 7.10; while the coefficient of variability for the ex-ultra-violet set was 44.80 and its control was 18.80. Table 3 illustrates the above comparisons.

Experiment 4. The effect of infra-red light, October 10 to October 25, 1928. On October 10, the second set of twenty lines of the clone used in experiment 3 were exposed to infra-red rays of wave length 6,5000 Å to 40,000 Å, at a distance of thirty inches from a Burdick Zoalite Z12 carbo-silicon arc. The twenty lines of animals were radiated for two minutes each day of a fifteen day experimental period. Twenty animals selected as were the experimentals, were kept under balanced selection as controls. Figure 10 is a graph of variation in actual number of fissions of the radiated set of animals, and of its control set of non-radiated animals for the fifteen day experimental period. The

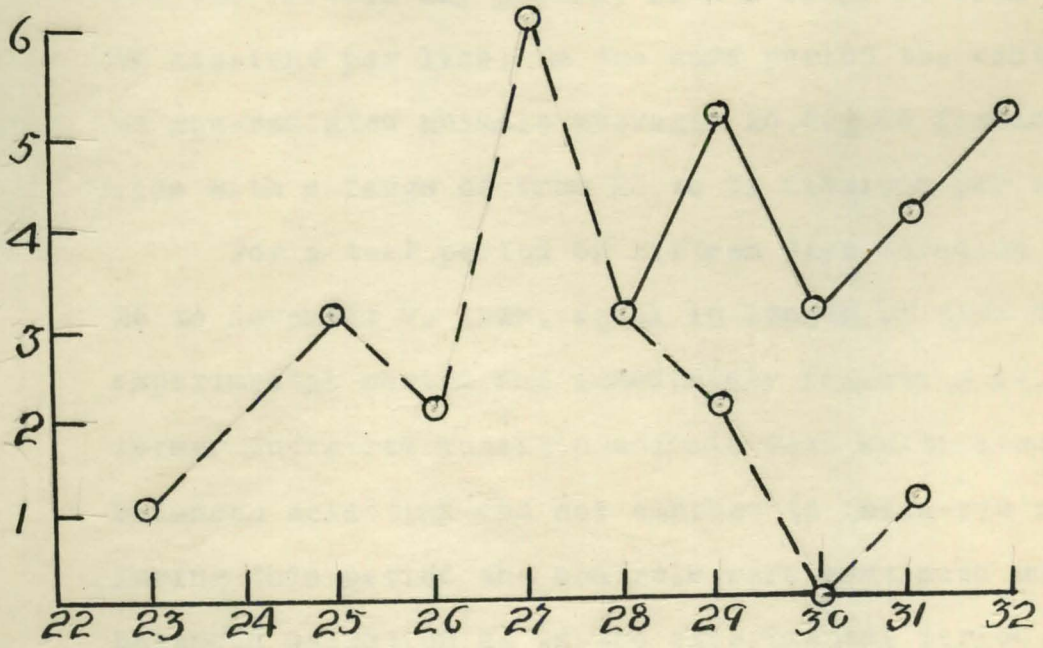

Fig. 10. Graph of variation in actual number of fissions of the radiated set of animals, and of its control set of non-radiated animals for the fifteen day experimental period. Ordinates represent occurence of lines; abscissae the number of animals per line.

Radiated animals averaged 30.15±.05 fissions per line for the fifteen day period, with a range of from 28 to 32 fissions per line; in the same period the control set of non-radiated animals averaged 26.65±.06 fissions per line with a range of from 23 to 31 fissions per line.

For a test period of fifteen days duration, October 26 to November 9, 1928, equal in length of time to the experimental period and immediately following it, the former infra-red radiated animals were cultivated under balanced selection and not exposed to infra-red rays. During this period the controls were continued under balanced selection as in the experimental period. During the test period the ex-infra-red lines averaged 13.60±.004 fissions per line, with a range of from 11 to 16 fissions per line; while their control lines for the same period averaged 13.30±.08 fissions per line, with a range of from 10 to 20 fissions per line. Figure 11 is a graph variation in actual number of fissions of the ex-infra-red and the control sets during the test period.

The arithmetical mean of the infra-red radiated set is greater by 3.50 fissions per line than the same

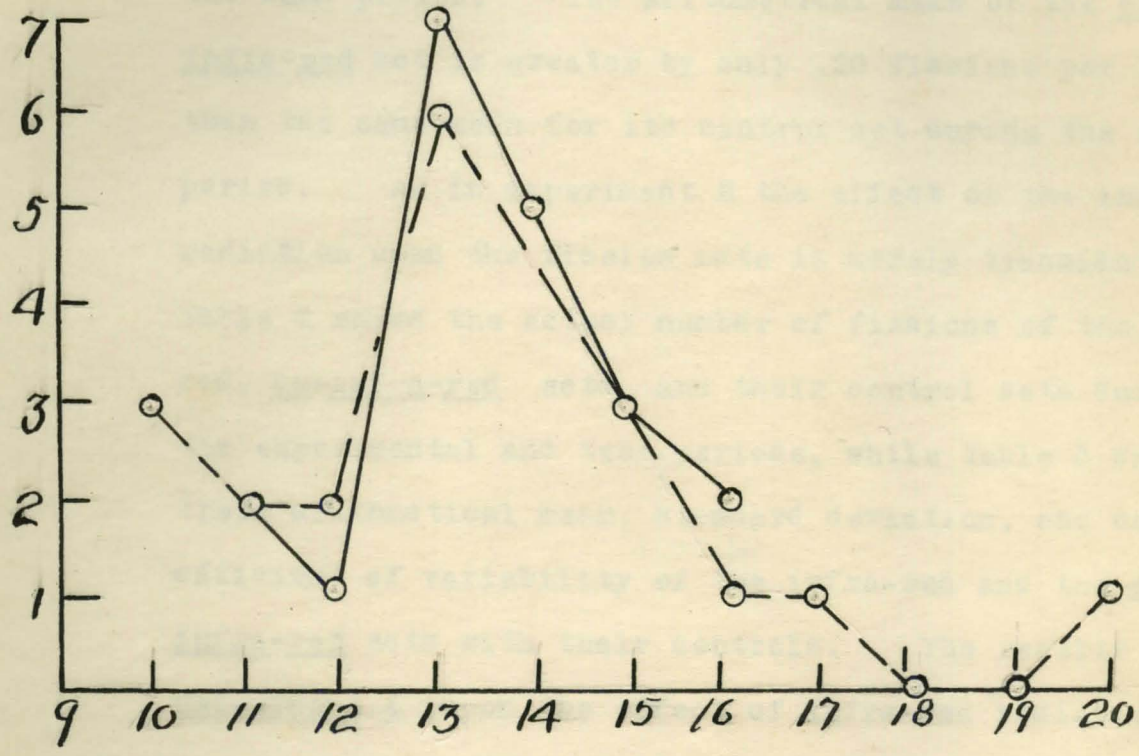

Fig. 11. Graph of variation in actual number of fissions of the ex-infra-red and the control sets during the fifteen day test period. Ordinates represent occurence of lines; abscissae the number of animals per line.

mean for its control set of non-radiated animals for the same period. The arithmetical mean of the ex-infra-red set is greater by only .20 fissions per line than the same mean for its control set during the same period. As in experiment 2 the effect of the infra-red radiation upon the fission rate is merely transient. Table 2 shows the actual number of fissions of the infra-red, ex-infra-red sets, and their control sets during the experimental and test periods, while Table 3 shows their arithmetical mean, standard deviation, and coefficient of variability of the infra-red and the ex-infra-red sets with their controls. The results of Experiment 4 (upon the effect of infra-red radiation) correspond with the results obtained in Experiment 2, which dealt with the same problem. (We are sincerely appreciative of the fine courtesy shown the Biological Laboratories of the University of Louisville by the Dick X-Ray Corporation of Louisville, Kentucky, which graciously loaned us the machines which were used as light sources in the experiments described in this paper.)

SUMMARY

In Experiment 1 during the experimental period of exposure to ultra-violet rays of wave length 1849 Å to 3900 Å the average fission rate was 8.35±.06 fissions per line. During a test period, equal in time to and immediately following the experimental period of radiation of the same set of animals, the average fission rate was 11.05±.09 fissions per line. In as much as the animals used in this experiment had been previously subjected to eighty days of fast selection obviously one should anticipate an accelerated fission rate following the cessation of exposure to ultra-violet rays. When the total number of fissions of the experimental and the test periods are contrasted with their controls (Table 3 and Table 1) for the same periods it becomes clear that the decrease between the average fission rates of the ex-ultra-violet set of lines and their controls during the test period, as contrasted with that difference during the experimental period, is due much more to a decrease in the fission rate of the control set than to the slight increase in fission rate of the experimental set and it should also be remembered that this slight increase in the fission rate of the ex-ultra-violet set over their

Table I

Table 1. Actual number of fissions of the individual number of lines of the ultra-violet and control sets, and of the infra-red and control sets during the experimental and test periods of experiments 1 and 2.

Table 2. Actual number of fissions of the individual lines of the ultra-violet and control sets, and of the infra-red and control sets during the experimental and test periods of the experiments 3 and 4.

Divisions	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	Total
No Lines In Control																					1	3	6	2	4	2	1	1						460
" " "Ultra Violet			1	1	1	2	5	4	4	2																								168
" " "Control											1		3	3	4	4	2	2	1															285
" " "Ex Ultra Violet		1					2	1	2	3	7		1	3																				220
No Lines In Control														1	2			2		2	5	3	2											408
" " "Infra Red																					1	1	1	5	7	2	1	1						455
" " "Control									1	1		1	5	3	5	1	1		2															244
" " "Ex Infra Red											1	3	6	5	2	2	1																	234

Table 2

No Lines In Control																								1	2	3	2	6	3	2		1		521
" " "Ultra Violet			1	1		5		3	3	3	3	1																						222
" " "Control											3	2	2	6	1	3	1	1		1														266
" " "Ex Ultra Violet	1	2		1	2	3	5	3	1	2																								127
No Lines In Control																								1	2	3	2	6	3	2		1		521
" " "Infra Red																														3	5	3	4	5	610
" " "Control											3	2	2	6	1	3	1	1		1														266
" " "Ex Infra Red												2	1	7	5	3	2																	272

Table 3.

The arithmetical means, standard deviations, and coefficients of variability of the experiment and control sets of lines of experiments 1, 2, 3, and 4, during both the experimental and test periods.

EXPERIMENT NUMBER 1	Arithmetical Mean	Standard Deviation	Coefficient of Variability
Ultra Violet Experimental	8.35±.06	1.96±.03	21.00
" " Test	11.05±.09	2.73±.04	24.70
" " Experimental Control	23.00±.06	1.80±.03	7.82
" " Test Control	14.25±.06	1.92±.03	13.40
EXPERIMENT NUMBER 2			
Infra Red Experimental	22.75±.06	1.90±.03	8.34
" " Test	11.70±.05	4.48±.07	12.64
" " Experimental Control	19.40±.01	3.04±.02	15.65
" " Test Control	12.30±.09	2.71±.04	22.20
EXPERIMENT NUMBER 3			
Ultra Violet Experimental	8.15±.08	2.45±.04	30.18
" " Test	6.10±.04	2.73±.04	44.80
" " Experimental Control	26.65±.06	1.89±.03	7.10
" " Test Control	13.30±.08	2.50±.04	18.80
EXPERIMENT NUMBER 4			
Infra Red Experimental	30.15±.04	1.42±.02	4.75
" " Test	13.60±.00	.113±.01	8.30
" " Experimental Control	26.65±.06	1.89±.03	7.10
" " Test Control	13.30±.08	2.50±.04	18.80

fission rate during exposure to ultra-violet radiation is probably due more to the fact that these lines had previously acquired a high average fission rate as the result of eighty days of selection.

In Experiment 3 during the experimental period of exposure to ultra-violet rays of wave length 1849 Å to 3900 Å the average fission rate was 8.15±.08 fissions per line. During a test period equal in time to and immediately following the experimental period of radiation of the same set of animals, the average fission rate was 6.10±.04 fissions per line. In as much as the animals used to start the twenty lines of this experiment were all of the same generation of the offspring of a single "wild" Paramecium Caudatum not selected either for fast or slow fission rate, the factor of differing initial hereditary fission rates is not here present. It is obvious that there is a progressive decrease in fission rate following exposure to ultra-violet rays of the above wave lengths in these lines.

The machine used as the source of the ultra-violet rays in Experiments 1 and 3 was of the mercury arc in quartz container type and produced rays of wave length

1849 Å to 3900 Å (184.9 μμ to 390 μμ) through the quartz container. The nucleus of Paramecium caudatum absorbs ultra-violet radiations of wave length 1850 Å (185 μμ). The bacterial content of the culture media was kept constant by the daily use of the technique described above. That technique consisted in a daily cross-inoculation of the culture medium on the slides; a daily washing of the selected animals in fresh culture medium; the daily sterilization of all utensils, immediately before and after each using in boiling distilled water, and by the transference of the selected animals to the new definitive slides before their exposure to ultra-violet radiation. Bacteria are killed by ultra-violet radiations of wave length 2900 Å or lower - Bovie, W. T. (41). The effect of ultra-violet radiations of the wave lengths used in these experiments upon the orgainc materials of the culture media was negligable for the short periods of exposure - Luckeish, M. and Pacini, A. J. F. (41). The experimental (radiated) and the test (ex-radiated) sets of animals with their corresponding control sets of animals were subjected to the same temperature conditions.

The results of Experiments 1 and 3 indicate that

ultra-violet radiations of wave length 1849 Å to 3900 Å have effected a change in the average fission rate of the individuals of these two clones of Paramecium caudatum and that this change, which is a reduction in the average fission rate, is permanent or heritable.

Experiments 2 and 4 in which the infra-red rays of a carbo-silicon arc were used indicate no permanent change in the fission rate within these two clones of Paramecium caudatum. The total number of fissions was greater in the radiated sets of animals than in their non-radiated control sets of animals during the experimental periods; but, the total number of fissions of the ex-radiated sets of animals and their control sets was approximately the same during the test periods (Table 1 and Table 2).

CONCLUSION

Ultra-violet radiations of wave length 1849 Å to 3900 Å (184.9 μμ to 390 μμ) caused a decrease in the average fission rates within the two clones of Paramecium caudatum used in these experiments and these new average fission rates persisted, in the absence of the experimental factor of the environment, for a period of time as long as that of the exposure to the radiation and through as many generations as occured during the experimental periods. In these two clones exposure to ultra-violet radiations of wave length 1849 Å to 3900 Å has therefore modified the average fission rate in the hereditary sense.

Infra-red radiations of wave length 6,500 Å to 40,000 Å (650 μμ to 4,000 μμ) causes an increased fission rate within the clone of Paramecium caudatum, as long as the environmental factor is present. When this environmental factor is withdrawn the more rapid fission rate of the infra-red radiated animals over their non-radiated controls disappears. In these two clones exposure to infra-red radiations of wave length 6,5000 Å to 40,000 Å have produced no heritable

effect upon the average fission rate within the clones.

Energy from light sources is accumulated in larger units with shorter wave lengths of light - Taylor, H. S. (58). The cell nuclues of Paramecium caudatum absorbs ultra-violet radiations of wave length 1850 Å (185μμ) - Bovie, W. T. (41). It is universally accepted that the cell nucleus is the activating medium responsible for all cellular division. Previous experiments have shown conclusively that the various amino acids of the cell nucleus absorb ultra-violet rays, although the specific wave lengths used in the experiments were not stated - Harris, F. I. and Hoyt, H. S. (28) and Koher, P. A. (36). The permeability of Paramecium caudatum to light reaches a maximum in the near violet range of the spectrum (1000 Å to 2,000 Å), and this permeability increases with the duration of the exposure - Packard, C. (46). The increase in permeability following exposure to the rays of radium (of wave length below 1,000 A) will cause an accelerated fission rate in paramecium, and in other cells and tissues - Packard, C. (46).

Ultra-violet radiations of wave length 1849 Å to

3900 Å, as used in the present experiments, resulted in a very apparent stable decrease in the fission rate within the two clones of Paramecium caudatum used.

The question "Can we change the living system by a modification of the environmental factors so that the modifications remain in later generations even after the modifying factor has been removed?" must be answered in the affirmative in so far as the two specific clones of Paramecium caudatum used in these experiments are concerned. In these clones at least it has been possible to modify the living system by a physical environmental factor, i.e., light radiation of wave length 1849 Å to 3900 Å so that the modification has persisted in later generations in the absence of the factor which called it forth.

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