[3] The observed probable error is computed from 36 degrees of freedom, the expected from about 600. The probability of a greater excess of the observed over the expected probable error is about 0.42 from the theory of sampling, and thus the observed excess of 7.83×10^{-8} over 7.69×10^{-8} has no significance.

[4] The weighted mean of the observed $v/2$'s is $2.2 \times 10^{-8} \pm 1.3 \times 10^{-8}$ (p.e.) in the refractive index. In comparison with its probable error, this mean does not differ significantly from zero. It will be seen that an extensive use was made of the method of least squares, which is rigorously correct only when the errors obey the normal law. There were, in all, 900 residuals in the least squares solutions, and these were found to conform to the normal law of errors, with no significant excess of large residuals. The tests were carried out by Pearson's X^2 method. The residuals were also tested for normality by the use of third and fourth moments.

[5] *Phil. Trans.*, **158**, 532 (1868).

IRRADIATION OF PLANT VIRUSES AND OF MICROÖRGANISMS WITH MONOCHROMATIC LIGHT. III. RESISTANCE OF THE VIRUS OF TYPICAL TOBACCO MOSAIC AND ESCHERICHIA COLI TO RADIATION FROM λ 3000 TO λ 2250 Å

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Communicated November 18, 1935

We have described in previous publications[1,2] the effect of monochromatic ultra-violet radiation down to λ 2650 Å (and 2537 Å) on a semipurified suspension of typical tobacco mosaic virus and a number of bacteria in the vegetative and spore stages, irradiated in the same suspension. It has since been possible to purify the virus suspension to a very much higher degree, producing a colorless and clear suspension, highly infective and with relatively little absorption at wave-lengths even as short as 2650 Å to 2250 Å in the dilution used. This high degree of purification has been attained by four simple procedures: (*a*) extraction of a crude extract from diseased plants by grinding and subsequently pressing out through cheesecloth, (*b*) subjecting the extract to a temperature of 65°C. for 15 min., (c) treatment (by stirring) with charcoal (Nuchar *W*) for 1 min., (*d*) filtration and refiltration with suction through hard filter paper.* The material was diluted to 5/100 with physiological salt solution for irradiation.

The bacterial work described in this paper has been conducted with a 15-hour agar slant culture of *Es. coli*. Previously the organism was grown in bouillon, as described in earlier publications, but it was found that even in extremely low concentrations bouillon absorbed highly wave-lengths below 2650 Å. In the present work the exposed suspension contained about 100,000 bacteria per cc. The details of the procedure of preparing

the bacterial suspension, as well as some minor changes in the apparatus, in the method of exposure and in the determination of the effect on the bacteria are described elsewhere.[4]

The infectivity of the virus suspension was determined by a modification of the primary local lesion method first introduced by Holmes[5] and later

TABLE 1

Exp. V_{11}. Nov. 1, 1934: Typical virus suspension, Nuchar (charcoal) treated; material clear and colorless, diluted 5/100 with physiological salt solution; exposure cell contents, 5 cc.; wave-length, λ 2650 Å; thermopile 11.2 erg/cm. of galvanometer deflection; operating temperature, 18°C.; inoculation of *Nicotiana glutinosa* immediately after irradiation, each run on 20 plants, one leaf per run on each plant; readings taken Nov. 7; all lesions counted.

TIME OF EXPOSURE, MIN.	ERG/CC. INCIDENT ENERGY	TOTAL NO. LESIONS IN 20 LEAVES	AV. NO. LESIONS PER LEAF	SURVIVAL RATIO
Control (3 sets)	0	658 (av. of 3 sets)	31.93	100
10	56,500	609	30.4	92.2
20	113,000	487	24.3	73.7
40	230,000	268	14.5	40.6
80	445,000	203	10.15	30.8
160	890,000	3	0.15	0.5

TABLE 2

All energy values are for 50% inactivation of 1 cc. of standard purified virus (typical tobacco mosaic) and *Es. coli* suspensions. Data are from complete inactivation curves. **E** 2250/**E** (virus) = Energy needed to inactivate at λ 2250 Å divided by energy needed to inactivate at the respective wave-lengths given in column 1 of table; for the bacteria (*Es. coli*), the reference wave-length is λ 2650 Å.

1	2	3	4	5	6
WAVE-LENGTH, Å	**E** (VIRUS), ERGS	**E** (*Es. coli*), ERGS	**E**(VIRUS LESS *Es. coli*), ERGS	$\frac{\textbf{E}\ 2250}{\textbf{E}\ (\text{VIRUS})}$	$\frac{\textbf{E}\ 2650}{\textbf{E}\ (\textit{Es. coli})}$
2250	27,700	8,500	19,200	1.000	0.498
2300	31,800	9,850	21,950	0.714	0.420
2400	58,000	9,400	48,600	0.477	0.442
2480	133,760	8,100	125,660	0.235	0.514
2537	117,000	6,500	110,500	0.237	0.638
2650	158,000	4,150	153,850	0.175	1.000
2805	300,000	7,000	293,000	0.092	0.693
2950–3050	500,000	13,000	487,000	0.056	0.320

statistically studied by Youden and Beale.[8] *Nicotiana glutinosa* was used as the test plant. The essential modification in the method consisted in using a measured area of each leaf selected and exercising extreme care to employ uniformity in rubbing the inoculum over this area. Each "run"—defined as one exposure of the suspension at any particular wave-length—was tested by inoculation of the exposed or control suspension on 16 to 20

leaves, each leaf on a separate plant, and also arranging that the selected leaves occupied all possible positions on the plants. For other runs, using the same suspension, other leaves on the same plant were utilized. On the average there were about 16 lesions per area with the control suspension at

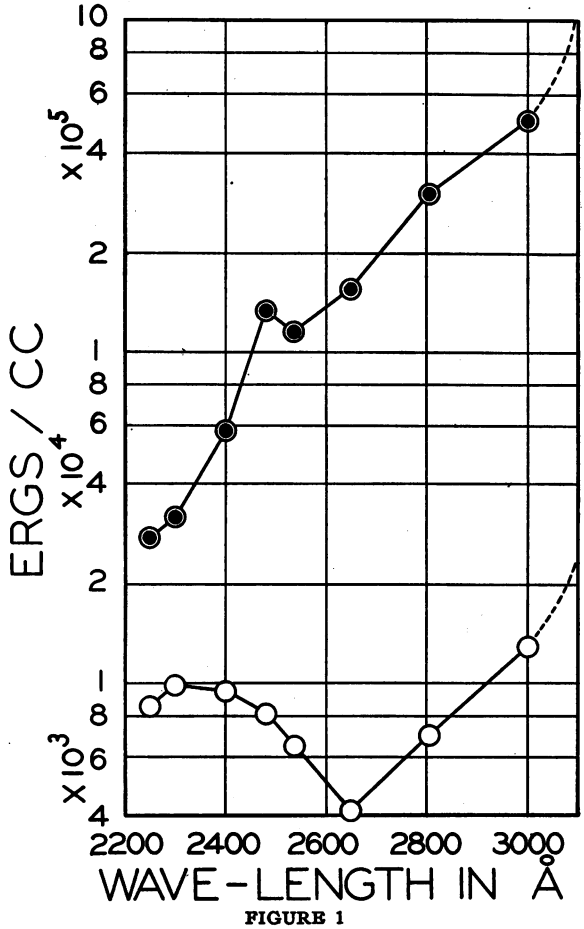

FIGURE 1

Incident energies in erg/cc. necessary to inactivate 50% of standard purified virus (●) and *Es. coli* (⊙) in the same suspension plotted against wave-length.

the virus dilution employed. Accordingly, the total "survival value" of the control for comparison was about 250 to 320 lesions per run. Altogether about 235 experimental runs were made in addition to about 30 preliminary tests. The data given in table 2 were obtained from more than 70,000 lesions. About 5 to 7 runs per wave-length were made and an inactivation curve was drawn for each wave-length. A typical protocol is

given in table 1. In table 2 there are given values for 50% inactivation of virus and bacteria. The data for the virus are averages of four complete (all wave-lengths) series or sets of data; i.e., the sensitivity for all the wave-lengths was obtained from one batch of material for each series. This was necessary since the absolute energy needed to inactivate the virus suspension varied with the previous history of the plants from which the diseased juice was prepared. However, the relative wave-length dependence as expressed in figure 3 was uniform for each single series. The points in the inactivation curve (Fig. 1), which we consider of special importance, that is, those around λ 2300 Å and 2650 Å were each separately investigated by four experimental series. It was found that 50% of the virus in 1 cc. at 2650 Å was inactivated by 158,000 ergs, whereas at 2250 Å, by 27,500 ergs. This order of magnitude in the sharp decrease toward the shorter wave-lengths, respecting the energy necessary to produce the inactivation effect, has been found in each case. The inflection of the curve around λ 2537 Å shifted sometimes toward 2650 Å, depending on the previous history of the material. The average of our energy values place it at 2537 Å, although it is quite possible that the maximum lies between λ 2537 and 2650 Å.

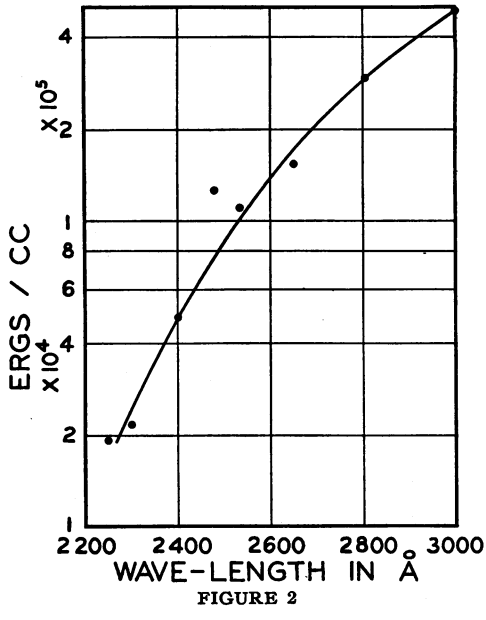

FIGURE 2

E (energy) virus less E bacteria for 50% inactivation in the same suspension plotted against wave-length. At wave-lengths longer than 3100 Å neither virus nor bacteria was inactivated by the energies used in these experiments.

In these experiments the virus has been found to be 40 times more resistant to λ 2650 Å than the bacteria, when irradiated in the same suspension (see table 2, column 4). This is, however, a significantly lesser value than the one given[2] for a less purified virus suspension. The difference is possibly explained by the assumption that the higher purification has liberated the virus which, in the case of the less purified virus suspension, may have been either clumped or attached to large particles.

Bearing in mind the relative resistance of this virus and of this species of bacteria to λ 2650 Å it is very striking and probably highly important that

the energy necessary to inactivate 50% of the virus at λ 2250 Å is not more than 3.1 times the energy necessary to inactivate 50% of the bacteria. This is brought out strikingly in figure 2. At wave-lengths longer than 3000 Å neither the virus nor this species of bacteria was inactivated by energies used in these experiments. In figure 3 there is presented the rela-

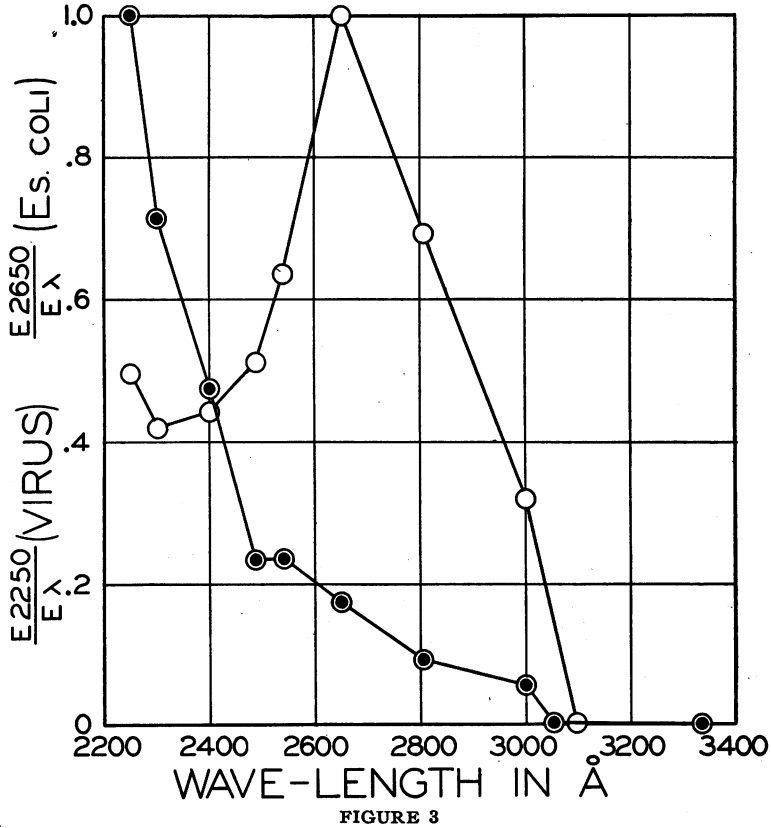

FIGURE 3

The curves show inactivation spectra for virus and bacteria, calculated for virus (◉) with reference to E (energy) at λ 2250 Å for 50% inactivation, and for bacteria (○) with reference to E at λ 2650 Å for 50% inactivation. These curves are given only to show the difference in the location of the maxima. For absolute energies of inactivation figure 1 should be consulted.

tive absorption curve which should have the shape of the reciprocal of the inactivation curve, that is, if all the absorbed energy is used in the inactivation of the material.. That the real absorption curve fits the form of the reciprocal of this inactivation curve (Fig. 1) is probable because the virus suspension in the dilution used has relatively little absorption and does not change in absorption measurably during irradiation. (See Warburg.[7])

Attention should be called to the resemblance of the wave-length de-

pendence of the inactivation curves of the virus as described in this paper to that of urease (Kubowitz and Haas[6]) and of pepsin (Gates[3]). Conclusions are, however, premature as long as we have no information (*a*) respecting the wave-length dependence of inactivation of proteins in general at the shorter wave-lengths, as well as (*b*) more information regarding the destruction spectrum of the virus of typical tobacco mosaic at wave-lengths below λ 2250 Å.

Summary.—The destruction spectrum of a highly purified suspension of the virus of typical tobacco mosaic as compared with the destruction spectrum of *Es. coli* in the same suspension is described for λ 2250 Å to λ 3000 Å. It has been found that: (*a*) at λ 2250 Å the amount of energy which is necessary to destroy 50% of the virus in 1 cc. is only one-fifth the amount required at 2650 Å, whereas (*b*) the energy necessary to inactivate bacteria is greater at λ 2250 Å than at 2650 Å.

This investigation has been carried out under the joint auspices (*a*) of the Committee on Radiation, Division of Biology and Agriculture, NATIONAL RESEARCH COUNCIL and (*b*) of the Graduate Committee of the University of Wisconsin and the Wisconsin Alumni Research Foundation. The inoculations were made chiefly by Dean F. McAlister and Tildon Easley, research assistants in virus investigations.

* Since this work was completed Stanley (*Science*, **81**, 1935) has reported the crystallization of the virus of typical tobacco mosaic, and he characterizes the virus as a protein.

[1] Duggar, B. M., and Hollaender, Alexander, *Jour. Bact.*, **27**, 219–239 (1934).

[2] Duggar, B. M., and Hollaender, Alexander, *Ibid.*, **27**, 241–256 (1934).

[3] Gates, F. L., *Jour. Gen. Physiol.*, **18**, 265–278 (1934).

[4] Hollaender, Alexander, and Claus, W. F., *Ibid.* (in press).

[5] Holmes, F. O., *Bot. Gaz.*, **87**, 39–55 (1929).

[6] Kubowitz, Fritz, and Haas, Erwin, *Biochem. Zeitsch.*, **257**, 337–343 (1933).

[7] Warburg, Otto, and Negelein, Erwin, *Ibid.*, **202**, 202–228 (1928).

[8] Youden, W. J., and Beale, Helen Purdy, *Contrib. Boyce Thompson Inst.*, **6**, 437–454 (1934).

CALCIUM AS A FACTOR IN THE NUTRITIONAL IMPROVEMENT OF HEALTH

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Read before the Academy, November 18, 1935

Investigations extending through successive generations of laboratory animals, with natural foods as the experimental variables, have shown[1-3] that a food supply which constitutes a permanently adequate dietary may still be capable of improvement by more scientific adjustment of the quantitative proportions in which the articles of food are consumed.