THE EFFECT OF pH ON INACTIVATION OF TOBACCO MOSAIC VIRUS BY X-RAYS*

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Previous experiments have shown that the sensitivity of chromosomes to x-rays can be reduced by treatment with ammonium hydroxide and that the protective action of the ammonium hydroxide increases with concentration.[6] This effect was attributed to the removal of positive charges on the chromosome surfaces. The experiments with tobacco mosaic virus reported here show that on the acid side of the isoelectric point where the molecules carry a net positive charge they are more sensitive to x-rays.

Experimental.—The purified virus, very kindly given to us by Dr. W. M. Stanley, was isolated by ultracentrifugation. One-tenth of a cc. of a distilled water solution containing 2.1 mg. of the virus was suspended in nine-tenths of MacIllvaine buffer at the proper pH and irradiated in paraffin-lined celluloid capsules. One sample of virus in buffer at pH 7.0 (reference point) was always irradiated at the same time as samples at other pH values. After irradiation the volume of solution was made up to 100 cc. and adjusted to pH 7.0 with the appropriate buffer solution. Controls received the same treatment except that irradiation was omitted. Experiments were limited to pH values from 2.2–7.0 since investigations of other workers[9] have shown that the virus is inactivated beyond that range.

The relative concentration of active virus was determined by the usual biological method of inoculating opposite halves of *Nicotiana glutinosa* leaves[4, 7] with the irradiated virus and unirradiated controls. For each sample 20 half-leaves were used.

In a preliminary experiment portions of a single sample of virus in distilled water were given different doses of x-rays and tested on half-leaves after diluting 1/1000. The curve of the logarithm of the number of lesions produced by irradiated virus as plotted against per cent of the unirradiated control is given in figure 1. The results fit the equation $Y = e^{-kx}$, where Y is the per cent unaltered virus, k a constant and x the dose in Roentgens. k has the value of 8.12×10^{-6} and the six points obtained fit the curve to within 1%. The sample of virus irradiated two weeks later showed inactivation within 2% of the values previously obtained. However, another sample of virus prepared in the same manner showed much greater sensitivity to x-rays. Curve *a* of figure 1 gives the results obtained with the first sample and curve *b* those of the second. The slopes of the two curves and therefore the x-ray sensitivity of the two samples of virus

differ by a factor of approximately 9. It was found necessary to use different samples of virus in the course of the first pH experiment. This, as will be seen later, did not influence the results obtained.

Results.—The counts of local lesions on pairs of half-leaves are listed in table 1.

TABLE 1

N. glutinosa LESION COUNTS FOLLOWING IRRADIATION AT DIFFERENT pH VALUES

	(1)		(2)		(3)		(4)	
pH	C_7	I_7	C_x	I_x	C_7	C_x	I_7	I_x
2.2	2015	1334	2025	1023	2430	2265	1002	613
3.0	2066	1344	2255	1387	2602	2457	1097	922
4.0	1213	867	1397	1017	(1)1154	1010	744	702
					(2) 433	442		
5.0	1854	1361	1537	1048	1486	1312	1308	1229
6.0	1854	1361	1707	1344	1611	1456	1445	1442

Column 1 gives the number of lesions produced by unirradiated virus at pH 7.0 (C_7) and irradiated virus at pH 7.0 (I_7), each being on halves of the same leaf of *N. glutinosa*. Similarly the spots produced on one set of half-leaves by unirradiated virus at different pH values is given by C_x (column 2) and the spots produced on corresponding half-leaves by virus irradiated at different pH concentrations by I_x. The controls in column 3 are compared with the irradiated samples in column 4.

The fraction of non-inactivated virus at pH 7.0 for any one run is given by I_7/C_7 and similarly at other pH concentrations by I_x/C_x. Letting $I_7/C_7 = S_7$ and $I_x/C_x = S_x$, then S_x/S_7 will give the survivors at pH_x as a fraction of the survivors at pH_7, and $1-S_x/S_7$ will give the efficiency of the irradiation in inactivating the virus at pH_x. The results of these computations are given in column *a* table 2. The efficiency of the x-rays at different pH concentrations may also be calculated from the data of (3) and (4) of table 1. The virus inactivation by pH alone will be given by $1 - (C_x/C_7)$ and this subtracted from $1 - (I_x/I_7)$ will give the x-ray efficiency of pH_x.

TABLE 2

THE RELATIVE EFFICIENCY OF X-RAYS AT DIFFERENT pH VALUES

	a	*b*
pH	$\left(1-\frac{S_x}{S_7}\right)$ 100	$\left[\left(1-\frac{I_x}{I_7}\right)-\left(1-\frac{C_x}{C_7}\right)\right]$ 100
2.2	+23	+32
3.0	+10	+10
4.0	− 2	− 7, +6
5.0	+ 7	− 6

Table 2 gives a summary of the x-ray efficiency in per cent of the inactivation at pH 7.0. At pH 4.0 a second set of inoculations was made with C_x and C_7 with the result shown in table 1. It is clear from this and

from the differences in the values obtained by the two methods described that errors as large as 13% may be expected.

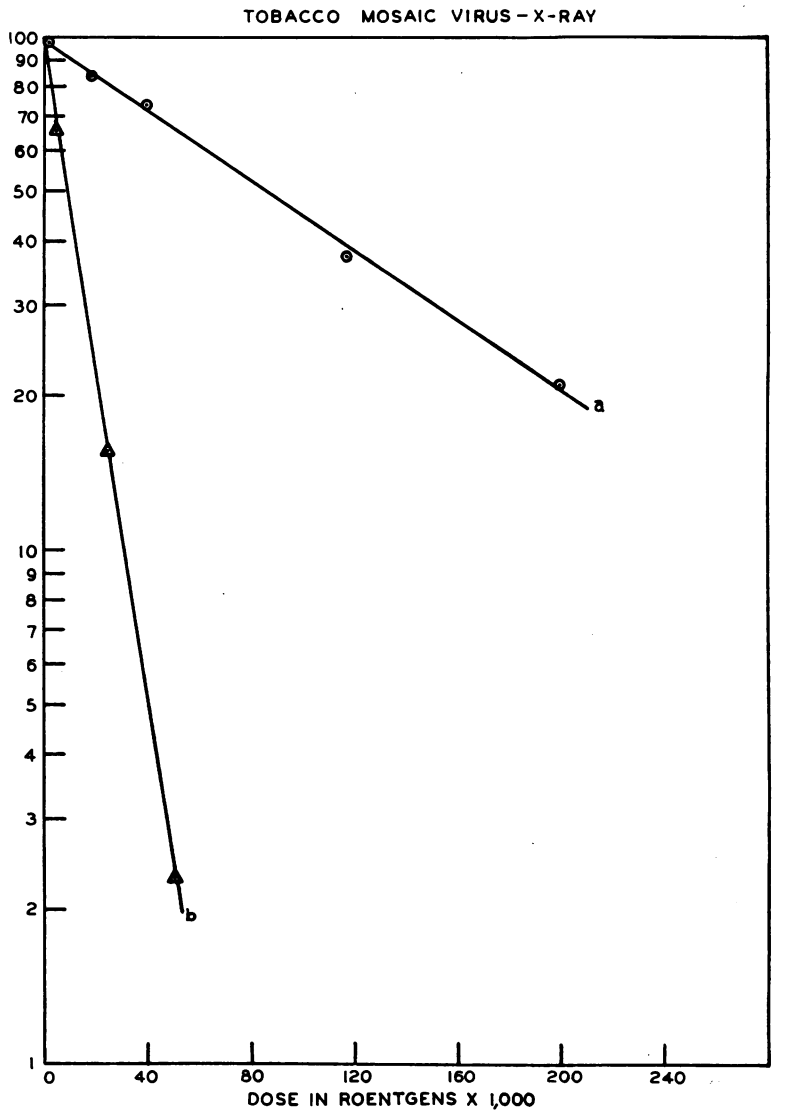

FIGURE 1

From examination of table 2 it appears that the efficiency of x-rays between pH 4.0 and 6.0 is equal to or less than the efficiency at pH 7.0. The differences are not large enough to be significant. However, at pH 2.2–3.0 the efficiency is significantly increased. To determine whether this might

have been an accidental result two more experiments were performed. In both of these the same sample of virus was used in determining inactivation at all pH values used. The results shown in table 3 are essentially similar to those of the previous experiment. At pH 2.2 x-rays are 25–30% more efficient in inactivating the virus than at pH 7.0, and at pH 3.4 the x-ray efficiency is greater by 10–15%. Between pH 3.4 and pH 7.0 there are no significant differences in the results.

TABLE 3

N. glutinosa LESION COUNTS AND X-RAY EFFICIENCY AT DIFFERENT pH VALUES

		$C_{2.2}$	$I_{2.2}$	$C_{3.4}$	$I_{3.4}$	$C_{5.6}$	$I_{5.6}$	$C_{7.0}$	$I_{7.0}$
Number	1	608	276	515	280	298	207	425	276
	2	220	110	266	156	322	207	284	199
$\frac{I}{C}$	1	0.454		0.544		0.695		0.650	
	2	0.50		0.586		0.643		0.678	
$\left(1-\frac{S_x}{S_7}\right)$ 100	1	+30		+16		−7		0	
	2	+26		+13		+5		0	

Gowen[3] studied the effect of x-rays on tobacco mosaic virus and found exponential survival curves. From these curves he calculated the size of the virus to be 7.5×10^{-18} cm.^{3}. He does not report differences in survival curves with different samples of virus. The sensitive volumes calculated from curves *a* and *b* of our experiments are 4.6×10^{-18} cm.^{3} and 4.2×10^{-17} cm.^{3}, respectively. Lea and Smith[5] irradiated dried and aqueous suspensions of tobacco necrosis virus with x-rays and found the results of both methods to fall on the same exponential survival curves. They mention that in different experiments different rates of inactivation were observed and the comparison mentioned above is made only with what they consider their best results. They found that tobacco mosaic virus in different states of aggregation gave essentially similar survival curves when treated with ultra-violet light. However, the experimental error of their observations was sufficiently large to make it impossible to determine whether their data indicated a response by elementary particles or aggregates as high as 4.

In the present experiments the shape of the survival curve is determined with an error no greater than 1–2% and can be explained only in terms of a response to x-rays by a single functional virus unit. Pirie[1] and Frampton[2] have presented evidence indicating that the virus particles may exist as aggregates. Filtration studies of the tobacco necrosis virus by Smith and MacClement[8] indicate the presence of aggregates in extracts containing this virus. The form of the survival curve we have obtained requires that at least one functional unit of the virus be inactivated for every ion pair or cluster produced in the virus elementary particle or aggregate. If aggregates exist, the following possible interpretations may be given to the data:

(1) There is only one functional unit in the aggregate; the rest is inert material.

(2) Aggregates are not permanent but are continually being broken down and built up from elementary units. As pointed out by Lea and Smith in this case the inactivation of particles in aggregates will proceed at the same rate as the inactivation of elementary particles and simple exponential curves will be obtained.

In both (1) and (2) we may assume that:

(*a*) The energy released by an ion pair or cluster within one elementary unit will inactivate only that unit.

(*b*) The energy released by an ion pair or cluster will inactivate more than one unit.

If after irradiation the virus is diluted before inoculation the number of aggregates will be decreased. Assuming condition (1), the apparent sensitivity of the virus will be independent of the state of aggregation and the spread of energy through the aggregate could not be detected. Under condition (2*a*) the sensitivity of the virus will be independent of the state of aggregation, while under condition (2*b*) the greater the aggregation at the time of the irradiation the greater the apparent sensitivity. The results obtained point to the latter hypothesis.

If it is postulated that the greater sensitivity to x-rays at pH 2.2 to 3.4 is due to a greater number of aggregates than at pH 3.4–7.0 the results cannot be adequately explained. One would expect a maximum sensitivity at pH 3.4, the isoelectric point, whereas the maximum observed is at 2.2. One would also expect that the samples of virus containing different amounts of aggregates would have different relative responses to x-rays at the various pH values. However, the same response was obtained although the sensitivity of the samples varied by a factor of 9. From these considerations it follows that the pH effect on x-ray sensitivity of the virus cannot be explained in terms of differing states of aggregation of the virus. A consideration of the charges on the suspended particles does provide an adequate explanation. The more acid the suspending medium, the greater will be the net positive charge on the suspended particles. The positively charged member of an ion pair produced by x-rays being of atomic size will be prevented from reaching the virus particle before losing its charge, while the negative ion being only of electronic mass will be able to penetrate to the virus and carry sufficient energy to inactivate the virus particle. The greater the net positive charge on the virus the greater will be its attraction for the free electrons produced by x-rays.

Conclusion.—1. Energy released by an ion pair produced within a virus particle may travel through that particle and inactivate one or more of the elementary virus units it may contain.

2. Electrons reaching the virus from the suspending medium have sufficient energy to inactivate the virus particle.

3. More of the electrons produced by x-rays in the suspending medium reach the virus particles when the latter carry a net positive charge than when the charge is negative. The greater the net positive charge the more electrons will be attracted.

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