SOME EFFECTS OF IODINE AND OTHER REAGENTS ON THE STRUCTURE AND ACTIVITY OF TOBACCO MOSAIC VIRUS

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The SH groups of denatured egg albumin give a pink color with nitroprusside (Heffter, 1907; Arnold, 1911) and reduce porphyrindin (Kuhn and Desnuelle, 1938). Native egg albumin does not give these characteristic SH reactions. Despite the fact that native egg albumin does not reduce porphyrindin, the SH groups of egg albumin or their precursors can be abolished by reaction of the native form of egg albumin with iodine (Anson, 1940; 1941).

Tobacco mosaic virus is an SH protein of the egg albumin type, since denatured but not native tobacco mosaic virus gives a pink color with nitroprusside and reduces porphyrindin (Stanley and Lauffer, 1939) and since the SH groups of tobacco mosaic virus, as shown by the present experiments, can be abolished by reaction of the native form of the virus with iodine. Iodine is the only reagent known to abolish the SH groups of egg albumin and tobacco mosaic virus by reaction with the native form of these proteins. The observations that denatured tobacco mosaic virus has SH groups and that these groups or their precursors can be abolished by reaction of the native form of the virus with iodine suggested the study of the effect of iodine on the activity of tobacco mosaic virus. It was found in the present work that the SH groups of tobacco mosaic virus can be abolished by iodine without any change in the activity of the virus, as shown by the number of lesions produced by a given amount of modified virus on *Nicotiana glutinosa* plants or by the symptoms produced in Turkish tobacco plants.

Tobacco mosaic virus has been inactivated by many different reagents, some of which are known to modify specific protein groups (Stanley, 1940). In the case of inactivation by formaldehyde, it was shown that the inactivation was accompanied by abolition of amino groups and that removal of formaldehyde was accompanied by an increase in free amino groups and by

partial reversal of the inactivation (Ross and Stanley, 1938). Although other viruses have been inactivated by formaldehyde, the structural changes brought about by formaldehyde were not established (Stanley, 1940). The formaldehyde experiments are the only ones in which the inactivation of tobacco mosaic virus has been associated with definite changes in protein groups by chemical tests on the modified protein. In no case has it hitherto been shown that tobacco mosaic virus or any other virus can be modified structurally by chemical procedures *in vitro* and still produce disease.

In the study of enzymes several cases have been found in which the enzyme structure can be altered without inactivation of the enzyme. The amino groups of pepsin can be acetylated without loss of the proteolytic activity (Herriott, 1934). Carboxypeptidase is active even in the presence of formaldehyde (Anson, 1937). Some of the SH groups of urease can be oxidized without any change in the urease activity (Hellerman, 1939).

The inoculation of Turkish tobacco plants with virus whose SH groups have been abolished with iodine results in the production of virus with the normal SH content. If the virus in the inoculum is not reduced within the living plant cells to virus with a normal SH content it must be concluded that iodine-modified virus causes the production, not of exact replicas, but of normal or unmodified virus. Such a result might be expected if virus with a normal SH content represents the nearest structure to the modified virus which may be synthesized within the plant cells due perhaps to some preexisting pattern. If, however, the inoculation of modified virus is followed by its reduction within the living plant cells to virus with a normal SH content it would be expected that the latter would stimulate the production of more normal virus. At the present time it is not known which of these alternatives represents the true course of events. It was found, however, that iodine-treated virus is not reduced to normal SH virus by a mash of normal tobacco plants. Unfortunately this is not absolute proof that such reduction does not take place in the living plant cells. Nevertheless it seems likely that reduction to SH of groups oxidized beyond S-S does not occur within the cells and hence that the present experiments provide an example in which a virus has been altered structurally without perpetuation of the structural changes in subsequent generations. Although at the present time it is impossible to assign definite reasons for the failure to perpetuate the structural change the results are important in connection with any consideration of the mechanism of virus reproduction.

If enough iodine is added to egg albumin (Anson, 1941) or to tobacco mosaic virus, not only are the SH groups abolished but the tyrosine groups are converted into di-iodotyrosine groups. When enough iodine is added

to tobacco mosaic virus to iodinate the tyrosine groups, the virus is inactivated. This result does not of itself prove that the inactivation is due to the change in the tyrosine groups and not to some other iodine reaction which occurs under similar conditions. However, it is known that when enough iodine is added to insulin (Harrington and Neuberger, 1936) or pepsin (Herriott, 1937) to iodinate the tyrosine groups, these proteins are likewise inactivated.

Whether or not it is possible to convert some of the tyrosine groups of tobacco mosaic virus into monoiodo or di-iodotyrosine groups without inactivating the virus is not decided by the present experiments.

Iodoacetamide at pH 8.0 under the conditions used in the present experiments abolishes few if any SH groups of tobacco mosaic virus, but nevertheless almost completely inactivates the virus. This result is of some interest for iodoacetamide has been regarded as a specific reactant for protein SH groups. It is hoped that the nature of the reaction which results in the inactivation by iodoacetamide will be elucidated in future work.

The present experiments raise such questions as whether all viruses, like tobacco mosaic virus, have SH groups which react with iodine but not with porphyrindin; whether in all cases oxidation of the SH groups fails to cause irreversible inactivation or indeed any change in the general character of the disease or in the type of virus produced in the infected plant; and whether all viruses can be inactivated by concentrated iodine and iodoacetamide. The fact that a virus variant was not produced by changes such as those described in the present paper does not mean that the production of variants by chemical treatment is impossible. It may be that the production of chemical variants must await the development of techniques for changing the amino acid content or arrangement of a virus without causing loss of virus activity, rather than merely changing amino acid groups such as SH. Experiments of the kind which have been done with tobacco mosaic virus in which the changes in both protein structure and activity are followed can, in case of necessity, be carried out with only a few milligrams of purified virus. It is to be hoped that similar experiments will be carried out with different viruses and also that different reactions will be used in the attempt to produce virus variants *in vitro* by definite chemical changes of protein structure.

EXPERIMENTAL

The Nitroprusside Test.—The nitroprusside test used in the present experiments is carried out as previously described in a solution of guanidine hydrochloride prepared from purified guanidine carbonate (Anson, 1941).

With recrystallized egg albumin about the same pink color is obtained with nitroprusside, whether 1 drop of 0.1 M cyanide is added or not. This small amount of cyanide suffices to combine with heavy metal impurities and does not cause any significant reduction of S-S to SH. With some samples of tobacco mosaic virus, however, an extremely weak nitroprusside test is obtained unless a drop of dilute cyanide is added. This indicates that some samples of tobacco mosaic virus may contain impurities which interfere with the nitroprusside test for SH groups.

The cyanide-nitroprusside test for S-S groups which are reduced to SH by cyanide is conveniently carried out as previously described (Anson, 1941) by adding 1 drop of 2 N cyanide to the protein in strongly alkaline guanidine hydrochloride solution and adding the nitroprusside 5 minutes later. The nitroprusside test carried out with no cyanide or with dilute cyanide which does not reduce S-S will be referred to as the nitroprusside test. When strong cyanide which can reduce S-S is added, the test will be called the cyanide-nitroprusside test.

SH Titrations.—The SH groups of denatured egg albumin can be estimated by allowing the protein to stand 45 minutes in neutral guanidine hydrochloride solution and then determining how much porphyrindin must be added to abolish the nitroprusside test (Greenstein, 1938). This method was applied to tobacco mosaic virus, and it was found that 1 cc. of 0.0006 N porphyrindin was required for 10 mg. of virus in order to abolish the nitroprusside test (Stanley and Lauffer, 1939).

The SH titration in guanidine hydrochloride solution has recently been modified in two ways. First, ferricyanide, tetrathionate, and *p*-chloromercuribenzoate are used as titrating agents instead of porphyrindin. Second, the titrating agent is added before the guanidine hydrochloride instead of 45 minutes thereafter (Anson, 1941). When purified guanidine hydrochloride is used, the same SH titration value for egg albumin is obtained whether ferricyanide is added before or after the guanidine hydrochloride. When the guanidine hydrochloride happens to contain impurities —which almost all commercial samples tested were found to contain—then low results are obtained by the original procedure because some SH groups are oxidized while the protein is standing in guanidine hydrochloride solution before the addition of ferricyanide. When ferricyanide is added to tobacco mosaic virus 45 minutes after the guanidine hydrochloride, different results are obtained by titrating the SH groups of different samples of virus even when purified guanidine hydrochloride is used, because some samples of virus themselves contain impurities which bring about the abolition of SH groups in guanidine hydrochloride solution. When guanidine hydrochloride of suitable purity is used and the titrating agent is added before

the guanidine hydrochloride, then 1 cc. of 0.00056 N ferricyanide, tetrathionate, or mercuribenzoate is needed to abolish the nitroprusside test of 10 mg. of tobacco mosaic virus, and all samples of virus give the same titration value. In the following experiments, SH groups are estimated by the ferricyanide titration method with ferricyanide added before the guanidine hydrochloride according to the directions previously described (Anson, 1941).

The SH groups of egg albumin (Anson, unpublished results) and of tobacco mosaic virus can also be estimated by measuring the blue color obtained when the proteins reduce Folin's uric acid reagent in neutral urea solution. The values obtained agree with those obtained by the ferricyanide titration.

Ross (1940) found that tobacco mosaic virus contains no methionine and a total amount of SH plus S-S sulfur which, within the experimental error, accounts for the total sulfur content of the virus of 0.2 per cent. The total SH plus S-S was estimated by titrating the SH groups in an HI hydrolysate, in which any S-S has been reduced to SH. The present SH titrations in guanidine hydrochloride solution confirm earlier results (Stanley and Lauffer, 1939) and show that all the sulfur of tobacco mosaic virus can be accounted for by SH alone.

Reactions with Iodine.—Tobacco mosaic virus prepared by differential ultracentrifugation (Stanley and Wyckoff, 1937; Stanley, 1937) has been treated with iodine under various conditions and the products tested for SH groups, for groups which can be reduced to SH by cyanide, for tyrosine groups by the Millon test, and for virus activity. The results are given in Table I. In the first experiments small amounts of iodine are added to neutral tobacco mosaic virus at 0°C., all the iodine is consumed, and the minimum amount of iodine is found which abolishes the nitroprusside test in guanidine hydrochloride solution.

0.5 cc. of iodine solution (prepared by diluting a stock solution of 0.1 N I_2 in 0.18 N KI) is added to 0.5 cc. of 2 per cent tobacco mosaic virus plus 0.1 cc. of a buffer consisting of equal parts 1 M Na_2HPO_4 and 1 M NaH_2PO_4. All the solutions are precooled in ice water. After this solution has stood 30 minutes at 0°C. and the solution gives no color with starch, the nitroprusside test in guanidine hydrochloride solution is carried out. The test is positive if the amount of iodine added is 1.5 times the amount theoretically needed to oxidize the SH groups found in denatured tobacco mosaic virus to S-S, and negative if 2.5 times the theoretical amount is added. Even when no test for SH groups is obtained, a strong test is obtained if the iodine-treated protein is first exposed to alkaline cyanide which can reduce S-S to SH.

If iodine is added to native egg albumin at 0°C. and in the presence of 1 N KI, the SH groups of egg albumin can be abolished by the addition of

1 cc. of 0.001 N iodine to 10 mg. of native egg albumin. This is exactly the amount theoretically needed for the oxidation of the SH groups in denatured egg albumin to S-S. If 2 cc. of 0.001 N iodine is added, then 1 cc. of 0.001 N iodine is consumed, as shown by back titration with thiosulfate (Anson, unpublished results). When 1 cc. of 0.00056 N I_2 (the theoretical amount) is added to 10 mg. of neutral tobacco mosaic virus at 0°C. and in the presence of 1 M KI, no iodine is used up in 30 minutes, as shown by back titration with thiosulfate.

TABLE I

Reactions of Iodine with Tobacco Mosaic Virus

Composition of reaction mixture 0.5 cc. virus solution 0.5 cc. I_2 solution 0.1 cc. PO_4 solution		Temperature	Time	Nitroprusside test	Cyanide-nitroprusside test	Millon test	Lesions per half leaf		Estimated amount of inactivation*
Concentration of virus solution	Concentration of I_2 solution						Iodine-treated virus	Control virus	
mg. per cc.	*N*	*°C.*	*hrs.*						*per cent*
20	0.00168	0	0.5	−	+	+	56.5	65.5	14
20	0.00280	0	0.5	−	+	+	70.0	79.0	11
10	0.01	37	2	−	+	+	35.7	35.4	0
10	0.04	37	2	−	−	+	29.1	29.2	0
10	0.04	37	8	−	−	+	79.2	104.5	24
10	0.04	37	24	−	−	+	35.8	46.0	22
10	0.05	37	2	−	−	+	28.8	34.8	17
10	0.06	37	2	−	−	+	25.4	32.2	21
10	0.06	37	2	−	−	+	31.2	47.7	35
10	0.03	55	0.5	−	−	+	15.3	30.1	49
10	0.04	55	0.5	−	−	+	11.6	22.0	47
20	0.1	37	2	−	−	−	2.5	30.3	92
10	0.1	37	2	−	−	−	4.5	34.5	87
10	0.05	60	1	−	−	−	0.0	20.2	100

* Differences of less than about 20 per cent are not regarded as indicating a significant difference in virus activity.

One cannot decide on the basis of our experiments alone why the SH groups of tobacco mosaic virus, unlike the SH groups of free cysteine and of egg albumin, do not react with dilute iodine in 1 N KI. It may be that the SH groups of tobacco mosaic virus or their precursors are less reactive than the corresponding groups of egg albumin. It is also possible that there are spatial obstacles to the ready formation of S-S groups in native tobacco mosaic virus (*cf.* Neurath, 1940).

One might suppose that the tyrosine groups of native egg albumin would react with dilute iodine in 1 N KI, even if the SH groups fail to react. Even free tyrosine, however, does not react with dilute iodine if the solution contains 1 N KI (Anson, unpublished experiments).

In the second series of experiments, enough iodine is added to abolish

the cyanide-nitroprusside test but not the Millon test, which is positive for tyrosine groups and negative for di-iodotyrosine groups. All the iodine is not absorbed, so the excess iodine is removed before the tests are carried out. When 0.05 N I_2 is added under the conditions chosen, the cyanide-nitroprusside test is negative, the Millon test is strongly positive, indicating that few tyrosine groups have been converted into di-iodotyrosine groups, and the virus activity is essentially unaffected as shown by the fact that the iodine-treated virus produces about as many lesions as an equal amount of untreated virus. As the amount of iodine added or the temperature is increased, the Millon test and the activity become weaker and insoluble protein is formed. Similar results can be obtained by adding 0.05 N I_2, that is, without increasing the iodine concentration, if the reaction is carried out for a day instead of 2 hours or if the solution is made more alkaline.

The experiments are carried out as follows. To 0.5 cc. of 1 per cent virus there are added 0.1 cc. of 1 M phosphate buffer at pH 6.8 and 0.5 cc. of iodine solution. The resulting solution is kept at the designated temperature and period of time in glass stoppered weighing bottles, then 0.5 cc. of thiosulfate of the same concentration as the iodine is added, and finally the mixture is made up to 5 cc. with water. For the color tests the protein is precipitated with 0.2 N trichloracetic acid, centrifuged, stirred up with 0.2 N trichloracetic acid, and centrifuged again. For the activity measurements, the solution is diluted 10 times with 0.1 M phosphate buffer at pH 7.0 and the virus activity compared with that of an equal amount of control virus by the half-leaf local lesion method on 20 or more leaves of *Nicotiana glutinosa* (Loring, 1937). The control virus is kept under the same conditions in the absence of iodine and at the end of the reaction tetrathionate instead of thiosulfate is added. At the virus concentrations used there is a direct proportionality between the virus activity and the number of lesions produced on *Nicotiana glutinosa* leaves although differences less than about 20 per cent in the lesion count are usually not regarded as indicating a significant difference in virus activity (Loring, 1937).

Although HI is a strong reducing agent, cysteic acid (RSO_3H) is not reduced to SH by HI under the conditions of the Baernstein HI hydrolysis of proteins (Kassell, 1940). Even when 0.04 N iodine is used to oxidize the SH groups of tobacco mosaic virus beyond the S-S stage, as shown by a negative cyanide-nitroprusside test, the protein on being dialyzed and then hydrolyzed with HI still yields as much cysteine as protein not treated with iodine. The oxidized groups are still reduced to SH by HI. This shows that the SH groups oxidized by iodine beyond the S-S stage are not oxidized as far as RSO_3H. We are indebted to Dr. A. F. Ross for carrying out the HI hydrolysis and estimating the cysteine content of the HI hydrolysate.

In the final experiment, a 1 per cent solution of virus is treated with an equal volume of 0.05 N iodine at 60°C. for 1 hour. The cyanide-nitroprusside and the Millon tests are negative, and the virus is completely inactivated. By carrying out the reaction at 60°C. instead of at 37°C.,

one avoids the formation of insoluble protein. If the protein is insoluble, one cannot be sure that the inactivation is due to the chemical change and not to the insoluble state of the protein.

SH Groups of Virus Produced in Plants Infected with Iodine-Modified Virus.—The following experiments show that the inoculation of Turkish tobacco plants with virus whose SH groups have been abolished by iodine is followed by the production of virus with the normal number of SH groups.

For the first experiment Turkish tobacco plants were infected with the virus partially inactivated by treatment with 0.06 N iodine at 37°C. as already described. Virus was isolated from the plants after they had been infected for 6 weeks and the SH of the virus was titrated with ferricyanide. The first time this experiment was carried out, virus was obtained whose SH groups were abolished by half the amount of ferricyanide normally required. When this experiment was repeated several times, however, the virus isolated from plants infected with iodine-treated virus always gave the normal ferricyanide titration value. Furthermore, tobacco plants infected with the virus which gave the low titration value also yielded virus with the normal titration value. It is not known why in one case and in only one case virus with a low titration value was obtained. It is possible that this one sample of virus became accidentally contaminated with impurities which interfered with the estimation of the SH groups.

The possibility existed that the iodine-treated virus preparation used to infect Turkish tobacco plants for the production of more virus contained a very small amount of virus which had escaped reaction with iodine, that this normal virus multiplied in the Turkish tobacco plants much more rapidly than the modified virus, and that the normal virus finally obtained had its origin in the small amount of normal virus which had escaped reaction with iodine. In one series of experiments, therefore, the iodine-treated virus was used to infect not Turkish tobacco, in which tobacco mosaic virus causes a systemic infection, but *Nicotiana glutinosa*, in which tobacco mosaic virus causes local lesions. When sufficiently dilute virus is used to infect such plants, each lesion is believed to contain only a single strain of virus, a fact which makes possible the separation of different strains of virus (Jensen, 1933; Kunkel, 1934). Turkish tobacco plants were infected by means of inocula prepared from single lesions previously obtained by rubbing iodine-treated virus over the leaves of *N. glutinosa* plants. The virus isolated from these Turkish tobacco plants was found to have a normal SH content.

In the first experiment, tobacco mosaic virus was treated with an equal volume of 0.01 N iodine for 2 hours at 37°C. as in the previous experiment with 0.04 N iodine. The

resulting virus gave a negative nitroprusside test but a positive cyanide-nitroprusside test. When tested against an untreated sample at a dilution of 10^{-4} gm. per cc. by the half-leaf method, the iodine-treated preparation gave an average of 35.7 lesions per half leaf and the control an average of 35.4 lesions per half leaf. If the lesions produced by the inoculation of iodine-treated virus had been caused only by virus which had escaped reaction with iodine, the number of lesions would have been greatly reduced by the iodine treatment. The virus treated with 0.01 N iodine was then used at a dilution of 10^{-6} gm. per cc. to inoculate the entire area of four leaves of a *Nicotiana glutinosa* plant. Five discrete and well separated lesions were selected and each was removed, macerated, and used as an inoculum for a group of four Turkish tobacco plants.

In a second experiment seven groups of four Turkish tobacco plants were infected by means of inocula prepared from seven single lesions obtained from virus almost completely inactivated by 0.1 I_2 instead of from virus not inactivated at all by 0.01 N I_2. It is extremely unlikely that this preparation contained any virus which had not reacted with iodine.

To 20 cc. of 2 per cent tobacco mosaic virus there were added 4 cc. of 1 M phosphate buffer at pH 7.4 and 20 cc. of 0.1 N iodine in 0.18 N potassium iodide. The mixture was kept at 37°C. for 2 hours, 20 cc. of 0.1 N thiosulfate was added, and the final solution was dialyzed overnight against cold distilled water in a shaking dialyzer. A precipitate of insoluble material was removed by centrifugation and found to contain 170 mg. of protein. The supernatant solution contained 0.2 mg. of virus per cc. which when applied to half leaves at a concentration of 10^{-4} gm. per cc. gave an average of only 2.5 lesions per half leaf. Since the untreated starting material when applied at a concentration of 10^{-4} gm. per cc. gave an average of 30.3 lesions per half leaf on the other halves of the same leaves the soluble fraction was about 90 per cent inactivated. The largely inactivated soluble fraction of the virus treated with 0.1 N iodine was used at a concentration of 10^{-4} gm. of protein per cc. to inoculate the entire area of four leaves of a *Nicotiana glutinosa* plant, in order to obtain the single lesions used to infect Turkish tobacco plants.

The character of the lesions produced by virus treated with 0.01 N or 0.1 N iodine and the course of the infection were the same as those observed when *Nicotiana glutinosa* plants are infected with untreated virus.

After 5 weeks the groups of Turkish tobacco plants infected from single lesions produced by virus treated with 0.01 N and 0.1 N iodine were cut and frozen as well as a group of Turkish tobacco plants inoculated at the same time with untreated virus. Several samples from each of the three groups were macerated and the virus isolated by the procedure involving differential centrifugation customarily employed in this laboratory. In all cases the virus finally isolated had the normal SH content as measured by ferricyanide titration and the normal specific activity as measured by the number of local lesions produced on half leaves.

The experiments which have been described show that the disease caused by virus whose SH groups have been abolished by iodine is not due to residual virus which escaped reaction with iodine. They show further that the iodine treatment does not produce a new variant. So far as one can tell by the tests used, iodine-treated virus brings about normal infection and the production of normal virus. The results do not permit a decision as to whether or not iodine-treated virus is reduced in the living plant to

normal virus before multiplication of virus takes place. It was proved, however, that a mash of Turkish tobacco plants does not reduce virus which has been oxidized by iodine.

The virus added to macerated Turkish tobacco leaves was treated with an equal volume of 0.04 N I_2 for 2 hours at 37°C., as previously described. After the addition of thiosulfate to destroy the excess iodine and dialysis, it gave a negative cyanide-nitroprusside test and a positive Millon test. 30 mg. of the iodine-treated, dialyzed virus was added to 20 gm. of a mash prepared by macerating by means of a meat grinder the fresh leaves of a normal Turkish tobacco plant. The mixture was allowed to stand overnight at room temperature and the juice was expressed and subjected to the purification process involving differential centrifugation customarily employed in this laboratory. The 18 mg. of virus which was isolated was found to give a negative cyanide-nitroprusside test.

TABLE II

Effect of Iodoacetamide on the Activity of Tobacco Mosaic Virus

Concentration of virus	Concentration of iodoacetamide	Temperature	Time	Lesions per half leaf		Estimated amount of inactivation
				Virus treated with iodoacetamide	Control virus	
mg. per cc.	*M*	*°C.*	*hrs.*			*per cent*
0.5	0.05	37	2	13.3	24.2	45
0.5	0.05	37	18	0.6	23.2	97
5	0.1	37	6	2.7	12.9	79
5	0.1	55	4	1.1	10	89

Reactions with Iodoacetamide.—Iodoacetamide at pH 9.0 abolishes 40 per cent of the SH groups of native egg albumin (Anson, 1940). Iodoacetamide (prepared according to Anson, 1939) was added to tobacco mosaic virus in 0.1 M phosphate buffer adjusted to pH 8 with NaOH, and the concentrations of the reagents and the time and temperature of the reaction were varied as shown in Table II. More alkaline solutions were not used in order to avoid inactivation of the virus by alkali. The virus in aliquot portions of the various preparations treated with iodoacetamide was precipitated and washed with trichloracetic acid, dissolved in neutral guanidine hydrochloride solution, and titrated with ferricyanide. In every case the titration value was the same, within 10 per cent, as that obtained from normal untreated virus. In all the cases the virus was partially inactivated by iodoacetamide. The exact degree of inactivation, as shown in Table II, depended on the exact conditions of the reaction and in one case was as high as 97 per cent.

In a report (Anson, 1940) of some preliminary experiments, it was stated

that tobacco mosaic virus could absorb iodine without being inactivated and that neither tobacco mosaic nor rabbit papilloma virus was inactivated by iodoacetamide. In the present experiments inactivation was brought about by the use of much more concentrated iodoacetamide.

Non-Inactivation by p-Chloromercuribenzoate.—*p*-Chloromercuribenzoate, an SH reagent introduced by Hellerman (1939), combines with the SH groups of denatured egg albumin and denatured tobacco mosaic virus. It combines with native egg albumin either not at all or very loosely (Anson, 1941). In the present investigation it was found that 0.1 per cent tobacco mosaic virus is not inactivated at room temperature in a neutral solution containing 0.001 N mercuribenzoate, an amount which would combine with all the SH groups of the virus if the virus were denatured.

SUMMARY

1. Denatured tobacco mosaic virus has a number of SH groups corresponding to its total sulfur content of 0.2 per cent. The SH groups were estimated by titration with ferricyanide, tetrathionate, and *p*-chloromercuribenzoate in guanidine hydrochloride solution and by reduction of the uric acid reagent in urea solution.

2. The SH groups of tobacco mosaic virus or their precursors can be abolished by reaction of the native form of the virus with iodine.

3. Tobacco mosaic virus whose SH groups have been oxidized beyond the S-S stage by iodine but whose tyrosine groups have not been converted into di-iodotyrosine groups still retains its normal biological activity as shown by the number of lesions it causes on *Nicotiana glutinosa* plants and by the characteristic disease produced in Turkish tobacco plants.

4. The inoculation of Turkish tobacco plants with active virus whose SH groups have been abolished by iodine results in the production of virus with the normal number of SH groups.

5. If enough iodine is added to tobacco mosaic virus or if the iodine reaction is carried out at a sufficiently high temperature, then the tyrosine groups are converted into di-iodotyrosine groups and the virus is inactivated.

6. Tobacco mosaic virus can be almost completely inactivated by iodoacetamide under conditions under which iodoacetamide reacts with few if any of the protein's SH groups.

7. Tobacco mosaic virus is not inactivated by dilute *p*-chloromercuribenzoate.

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