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Factors Affecting the Formation of Local Lesions by Tobacco Mosaic Virus

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[PLATE 13]

INTRODUCTION

The increase in knowledge of the virus diseases of plants has led to the possibility of attempting an investigation of the nature of the virus agent itself. It has been proved that various plants, notably *N. glutinosa*, react to inoculation with the virus of tobacco mosaic by the formation of necrotic lesions on the rubbed leaves. It has been shown, further, that the number of lesions formed increases with the concentration of the virus up to a definite value, which varies with the experimental conditions (Caldwell, 1933). At high concentrations of the virus, there is apparently not a sufficient number of susceptible areas on the leaves to allow of reaction to all the virus units present, and the number of susceptible areas becomes the limiting factor in lesion formation. At lower concentrations the number of virus units is the main factor involved.

Youden, Beale, and Guthrie (1935) have recently published a paper in which they show that all the available data on the formation of lesions by different dilutions of virus, obtained by different workers, can be fitted to a curve with the formula $y = \mathrm{N}(1 - e^{-ax})$.

It has been shown that many substances, when added to the virus, have the effect of reducing the number of lesions on inoculation, or of completely suppressing lesion-formation. This may be due to the action of the substances on the virus agent itself or to their action on the tissues of the plants, whereby the normal response of the tissues to infection is prevented. It is not always clear which of the two effects is responsible for the suppression of the lesions in some instances. The purpose of the present paper is to set on record a method which, it is hoped, could be used in a chemical study of the nature of the virus agent, the agent not necessarily being freed from the plant materials with which it is associated. By the use of this method, it should be possible to determine whether an

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inhibitor is acting on the virus agent itself, or on the host tissues or on both more or less simultaneously.

I have already shown that the curve indicating the number of lesions formed after rubbing leaves of *N. glutinosa* plants with different dilutions of the juice of tomato plants with aucuba mosaic has the form indicated in fig. 1.

In this the curve is at first more or less a straight line, while at the higher concentrations it tends to flatten out, as the number of susceptible areas becomes the limiting factor in the formation of lesions. The first part of the curve will the more closely approximate to a straight line.

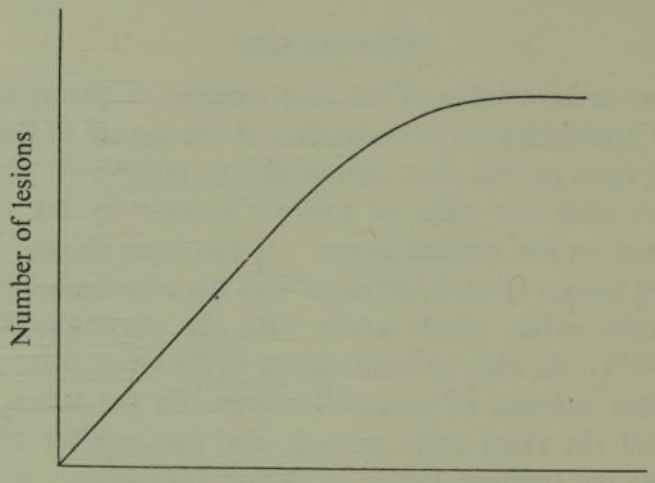

FIG. 1.

with a slope of 1, the more nearly every virus unit (or a constant proportion of the units present) reaches a susceptible area of the leaf. This would occur if the method of inoculation should distribute the virus units sufficiently over the leaf-surface. As the concentration of virus increases, the ratio SA/V steadily falls (where SA = susceptible areas and V = the units of virus), until there are no longer any available susceptible areas and additional virus has no appreciable effect on the number of lesions.

We have now to consider the position when a quantity of inhibitor is added which, by its action on the tissues of the leaf, reduces the number of susceptible areas to one-half, without affecting the virus agent. We assume that the same concentration of inhibitor is added to each concentration of virus. At the lowest concentration of virus, while the number of susceptible areas is reduced to one-half of that previously available, the ratio SA/V will still have a high value and the number of

susceptible areas will be more than sufficient for the available virus units. The second curve will therefore coincide with the first in the early stages. As the concentration of virus increases, however, the ratio SA/V will decrease more rapidly in this instance and the curve will become horizontal sooner than did that for untreated virus, the values on the horizontal portion of the curve being one-half those on the same portion of the curve for normal virus material.

As can be seen from the curves in fig. 2 the values on the curve for treated virus will never be less than half those for the untreated virus, and will vary from equality to one-half.

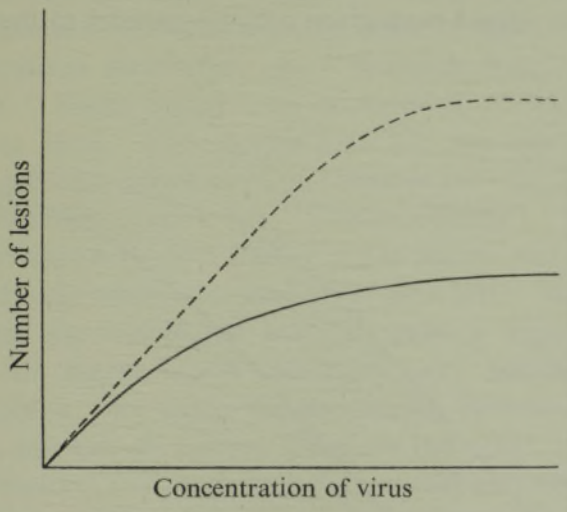

FIG. 2.

If the substance added to the virus reduces the number of lesions formed by its action on the virus agent itself and has no appreciable effect on the tissues of the host plant, then the curve for treated virus at different dilutions will take the form illustrated in fig. 3.

Here it is assumed that the inhibitor, under the conditions of the experiment, inactivates all the virus units at the lower concentrations. At these concentrations, therefore, no lesions are formed. As the concentration of virus increases, however, and the amount of inhibitor remains constant, the inhibitor will become saturated and an increasing amount of virus become available for lesion formation. The second curve, therefore, cuts the abscissa at some distance from the origin, and the distance between the point of intersection and the origin would increase with greater amounts of the inhibitor. As the concentration of the virus increases beyond the concentration at which the maximum number of lesions is obtained, then the effect of the inhibitor would cease to be

evident, and the curve for treated virus would coincide with that for control virus.

It should be possible where the effect of an inhibitor is well marked to demonstrate whether its effect is on the virus or on the plant tissues. When the inhibitor inactivates the virus, its effect should increase with decreasing virus concentration, the amount of the inhibitor being kept constant. When the inhibitor affects the plant tissues, however, the effects should decrease with decreasing virus concentrations.

This is in agreement with the views of Youden, Beale, and Guthrie (1935) that the data shown on fig. 3 of their paper indicate an effect of the inhibitor on the virus and not primarily on the host. A host effect, as they suggest, would have given a curve parallel to the abscissa.

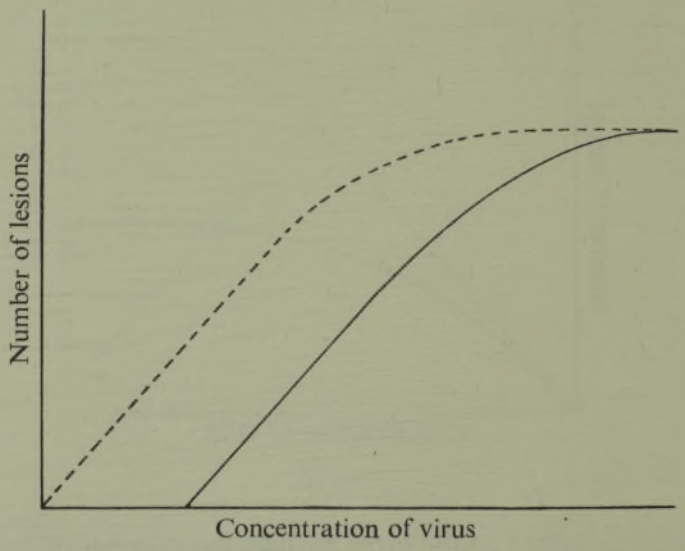

FIG. 3.

MATERIALS AND METHODS

The viruses used in the investigations discussed in this paper were those of aucuba mosaic and of ordinary mosaic of the tomato. The former virus is similar to, if not identical with, Johnson's *No.* 6 *virus*, while the latter is *virus No.* 1 of Johnson (1927). In the main, the work was carried out in the laboratories of this Institute, but some of it was done in the laboratories of the Rockefeller Institute, Princeton, New Jersey, during a visit there in the spring of 1935.

The virus material was sometimes prepared by the methods outlined in earlier papers of this series, *i.e.*, the infected material was macerated with twice its weight of water, filtered through muslin and then through

filter paper impregnated with fuller's earth. Crude juice from diseased plants was first frozen and subsequently filtered and used as the virus material, or in other instances the virus material was purified by the method of Vinson and Petre, as modified by the present author (1933). In a recent paper Petre (1935) supports the view that the modification increases the efficiency of the method. The method consists essentially in the precipitation of the plant proteins and of the virus agent itself by the use of lead acetate and the differential elution of the virus agent by suspension of the precipitate in M/15 di-potassium hydrogen phosphate. Where this material is used mention is made of the fact—and it was used primarily in experiments where only the minimum content was desirable.

For the preparation of virus material juice of diseased plants of the tomato *Lycopersicum esculentum* var. "Kondine Red", of the tobacco *N. tabacum* var. "White Burley", or, in America, of the tobacco variety "Turkish" was used. The inoculations for the assessment of virus content were made into leaves of *N. glutinosa* or into the cotyledons of the French bean *Phaseolus vulgaris*, var. "Golden Cluster". The *N. glutinosa* plants were cut down to five similar adult leaves and inoculations of treated material were made into one-half of each leaf. Into the other half control material was inoculated and this gave a measure of the susceptibility of the leaves which inevitably vary considerably in their reaction to rubbing. By using the proportion between the number of lesions on the one-half as against those on the other, a more accurate measure was obtained, it is suggested, than would have been available had the actual numbers been taken. Inoculations made on one-half leaves with the other half as controls, obviated the necessity of using measured doses of virus material, as each of the half-leaves similarly treated, would presumably be equally inoculated. In any method of lesion counting for assessing the number of virus units, the volumes of the inoculated materials must be fairly constant. Inoculations were made by rubbing the leaves with the tip of the index finger, or by the use of a small pad of muslin which had been saturated in the material to be examined.

Previous Work on the Effect of Inactivating Substances on the Virus Agent

Various workers have examined the effect of enzymes on plant and animal viruses and the results have, occasionally, been contradictory. Lojkin and Vinson (1931) found, for example, that none of the enzyme preparations which they used had the effect of inactivating the virus of

tobacco mosaic—(*T.V. No.* 1)—a rather surprising result. Emulsin, pepsin, and yeast extract did not, they conclude, reduce the infectivity of the virus agent in purified preparations, while trypsin, pancreatin, and papain did. Pepsin apparently was effective after an incubation period of several days. The present author has shown (1933) that pepsin in low concentrations, added to virus juice at a p_H of about 6·0 was ineffective. Pepsin at the same p_H, in high concentrations (*e.g.*, 2·5%) apparently does reduce the number of lesions formed on inoculation into *N. glutinosa* leaves (1935). This may be due in part to a protein effect such as is noted below. At a p_H of 2·5, however, pepsin at a concentration of 1% commercial pepsin preparation completely destroys the activity of the agent of aucuba mosaic if kept in an incubator at 35° C for 48 hours. When the p_H is returned to 6·0 and inoculations are made into *N. glutinosa* leaves no lesions are formed, and since pepsin is inactive at that p_H, and has been shown not to affect lesion formation (*see above*) it is fair to conclude that the virus agent has, in fact, been destroyed by the enzyme, since controls, held at the same temperature and at the same p_H, are almost equally effective before and after treatment. This is in accord with the data of Stanley (1934).

Lojkin and Vinson found that the enzyme effects were destroyed by boiling. They considered that the effect of trypsin, which was especially active in reducing the virus infectivity, was due to its hydrolytic action on the virus, rather than to a temporary inactivation of the virus by the enzyme. The evidence they adduce in support of the view is not very convincing. They exposed various virus preparations to trypsin at room temperature for periods varying from 1 hour to 22 days and found that these treatments reduced the activity of the virus agent to a greater or less extent. They then exposed similar juice to trypsin for 30 minutes at 0° C and found a reduction in the infectivity of almost 25%, which they considered as not significant. It must be noted, in this connexion, that these authors give surprisingly low infection percentages for their control plants inoculated with undigested juice. In all their experiments less than 100% infections of the inoculated tobacco plants was observed, when inoculated with the purified juice to which no enzyme was added, and the number of lesions formed on the leaves of *N. glutinosa* plants by similar material was of the order of 10–20. This would suggest that the methods of purification were not very satisfactory, since plant juice prepared by the method outlined in the papers of this series induces the formation of many hundreds of lesions on the inoculated leaves of *N. glutinosa*.

The general inference that Lojkin and Vinson drew from their results

was that the enzymes acted on the virus as they would on protein substances, in other words, the effect on the virus was proteolytic.

In a paper published in 1933 I showed that the effect of trypsin on the virus was not proteolytic and that heating the trypsin-virus mixture at 70° C for 15 minutes, destroyed the enzyme, and resulted, on inoculation into the leaves of *N. glutinosa*, in the formation of necrotic lesions in approximately as large numbers as were induced by the original virus material. In a subsequent note Vinson (1934) has expressed his agreement with this conclusion. I attributed the absence of lesions after inoculation into the leaves of *N. glutinosa* to the inactivation of the virus, probably by adsorption on to the enzyme, and to the proteolytic action of the enzyme on the leaf tissues of the inoculated plants. No evidence was obtained that the enzyme, even in concentrations much greater than those used in the experiments with virus juice had any toxic effect on the tissues of the rubbed leaves. In a recent paper Stanley (1934) has concurred with the view that the effect of the enzyme on virus is not proteolytic. With some of my conclusions he has, however, disagreed and a subsequent section of this paper deals specifically with a discussion of the problem. The main conclusions at which Stanley arrived are as follows:

" If the virus were actually split or digested by trypsin, as first suggested by Vinson, reactivation by heat would be improbable . . . if the inactivation were due to proteolysis one would expect pepsin rather than trypsin to be an active proteolytic agent. . . . Since the virus in the trypsin-pepsin digest gave an average of about 77% as many lesions as did the virus in the untreated control solution, it is obvious that much of the virus actually was regained by digestion of the trypsin with pepsin." (This confirms the conclusion of my earlier paper.) . . . " If the loss of virus activity on the addition of trypsin is due to proteolysis it should take place only at hydrogen-ion concentrations at which trypsin is active . . . Trypsin has its maximum proteolytic activity between p_H 7·5 and 9·5 and is inactive below 6·0." (This argument might equally well be used against the author's own conclusion that " trypsin . . . present at the time of inoculation or within 30 minutes thereafter, apparently affects infected cells, possibly by killing them " since the cell-sap of *N. glutinosa* leaves has a p_H of the order of 5·5, . . . definitely on the acid side.) Stanley's conclusion is that " the effect of trypsin in causing a loss in virus infectivity is due chiefly to its action on the plant ".

The data that Stanley adduced on the diffusion rate of trypsin are probably not of much real value in a discussion on the existence of a virus-trypsin complex as it is arguable, as Stanley himself suggests, that the amount of trypsin was so much greater than the amount of virus

present that the residual trypsin (assuming the existence of the complex) was insufficient in quantity to allow of its being considered as acting alone in the diffusion system. Stanley's own data published in Table VII of his paper suggest that the inactivation of the virus does enormously decrease with dilution, as tested by him on *P. vulgaris* leaves and that this is good presumptive evidence of an effect of the trypsin on the virus. At the same time it is clear that his data with *N. glutinosa* suggest that some effect on the plant-tissues is evident. In effect, Stanley has definitely confirmed my finding that the effect of trypsin on the virus is not proteolytic and that there is some effect on the virus, as opposed to the effect on the plant tissues. Which of these two effects is the more important, seems to be still a matter for discussion.

Experiments on the Inactivatory Effect of Normal Serum

The preparation of immune-sera by various workers (Beale, 1934; Birkeland, 1934; Chester, 1934, 1935) has greatly facilitated the separation and the identification of viruses by serological methods. The use of these sera, as specifically diagnostic material, has to some extent obscured the fact that many protein materials have at various times been used as inactivators of virus. It has been reported by various workers that the addition of proteins and similar substances has resulted in the reduction in number of spots following inoculation of a virus suspension into *N. glutinosa*. With a view to ascertaining whether the effect of the protein was primarily on the virus or on the plant tissues a series of experiments was carried out. In the earlier experiments, it was shown that 1% casein (soluble), 1% albumen, and 1% peptone in the virus suspension were effective in reducing the number of lesions to a greater or less extent. These substances were added to the virus juice and the mixture was left overnight on the laboratory bench before being used as inoculum. Actually, it is possible, as is described below, to inhibit the formation of lesions by the treatment of the leaves after inoculation with some of these and other substances.

After the preliminary experiments of a general nature, series of experiments were set up to ascertain whether or not the effect of the native proteins was primarily on the virus, or on the plant, as judged by the different effects with different concentrations of virus.

In most of the earlier work recorded in previous papers of this series whole leaves of plants of *N. glutinosa* were inoculated by rubbing, with the tip of the index finger, equal volumes of virus suspension on to each leaf. It was found that there was considerable variation from plant to

plant and even between successive leaves on the same plant. The underlying causes of these differences are obscure but it is clear that they must be associated with the individual leaves. In an attempt to obviate the difficulties which arise by reason of these differences half-leaves were rubbed with the virus juice and were considered separately. It was found that in the main the right-halves of the leaves developed rather more lesions than did the left-halves, presumably because of the different treatment to which they, being more easily rubbed, were subjected. The difference might be as great as to give 75% the number of lesions on the left-halves that were developed on the right-halves. It was, therefore, found desirable to alternate the halves which were inoculated with juice after any one treatment and to use the other half-leaves for inoculation with the control solution. In this way, the following technique was evolved. The *N. glutinosa* plants were selected as soon as they had developed five mature adult leaves of approximately equal size, and the other leaves were removed. Thereafter a notch was cut in one or other side of each leaf, alternately on the left- and right-halves. Inoculations were made, with the untreated material on the un-notched side of each leaf, while on the other side, the corresponding material after treatment was applied. Thereafter, the *proportion* between the numbers of lesions induced by the treated as against the untreated was taken as a measure of the effect of the treatment.

In the first experiment the whole leaf was rubbed with virus material—juice of infected Turkish tobacco plants infected with tobacco *virus No.* 1 (*T.V. No.* 1). The tissues were macerated with twice the volume of water in the usual way and cleared by alternate freezing and thawing for a few days. The supernatant liquid was used as a source of material. This work was carried out at the Rockefeller Institute at Princeton, N.J. After the whole leaf had been rubbed one-half of each leaf was coated with normal rabbit serum, applied immediately with a camel-hair brush. There was a reduction in the number of the lesions on the treated half-leaves to about 40% of those on the untreated halves. In the next experiments the normal rabbit serum was mixed with an equal quantity of virus juice and similar juice was diluted to one-half with water to act as the control material. The mixtures were left on the laboratory bench for 2 hours and were then kept overnight frozen in a refrigerator. Inoculations were made by rubbing the juices on to half-leaves, using pads of cheese cloth soaked in the materials. No lesions whatsoever appeared on the half-leaves rubbed with the juice containing the 50% serum, while large numbers of lesions appeared on the other half-leaves. In another experiment only 2% normal rabbit serum was added to the virus juice,

with appropriate controls, to which 2% extra water was added. In this case, the reduction in the number of lesions was approximately 50% of those developed on the control half-leaves.

It was clear from these experiments that 2% normal rabbit serum was rather too low a concentration to give complete inhibition in the use of crudely purified virus juice under the condition of the experiment. In the next experiment 10% of normal rabbit serum was added to diseased tobacco juice diluted to 1/5, 1/25, 1/125, 1/625, and to undiluted juice. After being kept overnight in a refrigerator, the juice was inoculated on to the half-leaves of *N. glutinosa* plants, when it was found that the inhibition was approximately 60%, 90%, 95%, 100%, and 100% in the dilutions 1/1, 1/5, 1/25, 1/125, 1/625, respectively. From the data, it was clear that increased dilution was associated with increased inhibition, with constant amounts of inhibitor, indicating that the effect of the inhibitor (some component of the normal rabbit serum) was in this instance acting primarily on the virus, if the argument set out in the earlier part of this paper be valid. It was noticed that there was some precipitate thrown down in those tubes which contained the higher concentrations of virus. To obviate so far as possible the removal of the virus mechanically by this precipitate, healthy tobacco juice was added to the serum before the diluted serum was mixed with the virus-containing material. Since the protein content of this healthy virus juice was far in excess of the much diluted virus juice, the formation of a precipitate between the serum and the plant-substances present in the virus material was largely or entirely prevented. Actually, cleared juice, prepared by filtration through fuller's earth or by the more elaborate method detailed above, rarely, and then only in the highest concentrations, gave any visible precipitate at all.

Experiments with Horse Serum

The preparation of normal rabbit serum involved a considerable amount of work, which was not entailed if normal horse serum produced commercially, was substituted for the normal rabbit serum. This was bought already prepared in 10 cc bottles.

The prepared serum contained traces of antiseptic substances (*e.g.*, tricresol) but there was no evidence, on comparison with normal rabbit serum, that this had any specific effect on either the virus or the tissues of the host plant.

In the preliminary experiment 1% normal horse serum was added to filtered juice from tomato plants with aucuba mosaic (strain Y) in con-

centrations of 1/1, 1/2, 1/4, 1/8, 1/16 and these were left overnight on the laboratory bench. Inoculations were made on the half-leaves of plants of *N. glutinosa*, using small muslin pads soaked in the treated juice or in the appropriate control juice. After inoculation, the leaves were washed with a stream of water to remove excess of the inoculum. In the course of the next few days lesions appeared on the treated plants, and it was found on the fifth day, that there was no recognizable inhibition in the three highest concentrations of juice (*i.e.*, 1/1, 1/2, 1/4) while there was an inhibition of lesion formation to the extent of 35% in the 1/8, and to 66% in the 1/16. This is, again, an instance of the effectiveness of a given concentration of serum being evident at low concentrations of virus, while in the presence of excess virus no evidence of inhibition was obtained. Since 1% normal horse serum had no evident effect on lesion formation at the higher concentrations of virus, the effect at lower concentrations could hardly be attributable to effects on the host tissues. The action of the serum must, therefore, be mainly on the virus itself. To establish this point a further experiment was set up in which 90% juice and 10% normal horse serum were tested against 90% juice and 10% healthy juice, and 10% juice, 10% serum, and 80% healthy tomato juice against 10% juice, 10% water, and 80% healthy tomato juice. 10% serum reduced the production of lesions by 90% virus juice by only 20% whereas the same concentration of serum completely inhibited the formation of lesions at the lower concentration of virus. The juice in this experiment was purified by the elution method, outlined above, and the M/15 di-potassium hydrogen phosphate eluate was adjusted to p_H 7·2.

A large number of experiments has been carried out with aucuba mosaic juice, both purified and unpurified, and with larger or smaller quantities of serum added to different concentrations of the virus. To ensure that the protein content of the inoculum should be reasonably high the procedure has been to make up the serum or other test substance in double concentration in undiluted healthy tobacco juice, extracted and filtered through fuller's earth.

Invariably, with concentrations of serum around 5%, there was a progressively greater reduction in the proportion of the lesions induced by the untreated juice, as the concentration of the virus was reduced. It is abundantly clear, therefore, from these and from other experiments of Chester (1934) that normal serum does reduce the number of lesions induced on *N. glutinosa* and other hosts but it is suggested that the data here obtained and also those recorded by Chester indicate that the main action of the serum is on the virus itself.

Experiments with Trypsin

Similar experiments to those outlined above have been carried out with virus juice, prepared in various ways, to which small quantities of trypsin had been added. It was found in preliminary experiments that a 2% trypsin suspension washed over halves of leaves inoculated with virus juice greatly reduced the number of lesions on the treated as compared with the untreated half. This effect was most marked when the trypsin was applied immediately after inoculation when the lesions on the treated half-leaf were reduced to less than 10% of those on the other portion, and was progressively less marked as the interval between inoculation and treatment was increased to about an hour, when no reduction in lesion-number was noted. The reduction in the lesion-number when the trypsin-suspension was applied after a short period may be attributable to the effect of the enzyme on the injured tissue or on the virus agent itself, before the latter had become incorporated with the host tissue.

When 0·25% commercial trypsin (B.D.H.) was added to a series of dilutions of virus juice and the mixtures were kept at room temperature for 24 hours before inoculations were made as described for the serum-mixtures, the inhibition due to the trypsin did not show the marked effect of that due to the serum. At a dilution of 1/2, *i.e.*, with 50% healthy tobacco juice, and 0·05% trypsin the reduction in lesions on the treated half-leaves was 22% on an average, and at a dilution of 1/6 with the same enzyme concentration, 82%, while it rose to over 90% at 1/8, 1/54, and 1/162. This progressive increase in inhibition with decreased concentration of the virus has been shown to be indicative of an effect of the inhibitor on the agent, while the fact that the effect is not so pronounced as that of the serum, for example, would suggest that there is also an effect of the enzyme on the tissues. It is suggested, however, that while this effect is admitted, the fact that progressive increase in the inhibition with decreased concentration, which has been shown both by Stanley's experiments and by mine, indicate an effect of the trypsin on the virus, which as I have clearly shown, need not necessarily be proteolytic.

Experiments with Silver Nitrate

A series of experiments has been carried out on the effect of the addition of silver nitrate on the production of lesions by aucuba mosaic. Chester (1935) has shown that silver nitrate does not appear to affect the antigenic properties of the virus of tobacco mosaic (T.M. *No.* 1). Various concentrations of virus material were made by extracting, under pressure,

the juice of tomato plants infected with aucuba mosaic and then diluting the infected juice with expressed healthy juice. In this way it was hoped to keep the total amount of plant materials in the juice at about a constant level. The concentrations of virus were 1/2, 1/20, 1/200, 1/2000, and to each group were added constant amounts of silver nitrate to give concentrations of 0·1%, 1·0%, and 0·25%. The controls and the treated materials were allowed to stand for 24 hours on the laboratory bench and were then inoculated, as indicated above, the control material into one half-leaf and the corresponding treated material into the other. After a week the plants were examined when it was found that the silver nitrate had had the following effect on the formation of lesions.

In the series of dilutions to which 0·1% $AgNO_3$ had been added there was a slight reduction in only the 1/2000 concentration. This amounted to 40% on *an average of ten leaves* while in the higher concentrations of virus no effect of the silver nitrate was detectable. In the series to which 1·0% $AgNO_3$ was added the effect of the salt on the leaf-tissue was so pronounced that, even though the surplus inoculum in these, as in all the other experiments, was washed off with a stream of running water, the tissues were definitely affected by the inoculum and no virus necrotic lesions appeared on any of the treated plants at any concentrations of the virus.

In the third series the effect of the silver salt was very striking. In the lowest virus concentration there was a complete inhibition of lesion formation on the half of the leaves inoculated with the treated juice, while at 1/200 concentration only a single or at most a few lesions were formed the inhibition was more than 95% complete. At 1/20 concentration, however, considerable numbers of lesions were formed on the side inoculated with treated juice. The inhibition was of the order of 70%. In the highest concentration of virus, viz., 1/2 the lesions were so numerous on both sides of the leaf that it was impossible to demonstrate any inhibitory effect of the treatment at all. Four photographs showing the appearance of the treated leaves are given in Plate 13. In all these and similar experiments carried out with silver nitrate there was a copious precipitate in treated materials. This was thoroughly shaken up before inoculations were made and was inoculated on to the leaves under experimentation.

Discussion

It has been shown that the efficiency of an inhibitor acting on the virus itself should increase with decreasing concentration of virus while that of

an inhibitor acting on the plant tissues should decrease with decreasing virus concentration. While no attempt is made to explain the action of the three substances used in the experiments, viz., trypsin, animal serum, and silver nitrate on the virus, it is suggested that the results obtained with them show that they have a definite effect on the virus present in the inocula.

No matter what that effect be due to, the method outlined in this paper, if used with substances the reactions of which on proteins and other substances are known, furnishes a means of studying the chemical nature of the virus agent, even if that agent were not necessarily in a pure state. The use of this method should make possible a direct chemical examination of the virus agent in an infected juice.

Summary

Many viruses produce local lesions when the plant juices containing them are rubbed on the leaves of appropriate plants. It is known that this effect may be reduced or even abolished by the addition of certain substances, *e.g.*, normal serum, to the virus-holding juice. The action of these inhibitory substances may be either directly on the virus itself, *e.g.*, by neutralizing its infectivity, or indirectly on the leaves of the rubbed plant, *e.g.*, by reducing their susceptibility; and it is a matter of some moment to distinguish between these two modes of action. A method is suggested whereby the distinction can be made.

The action of various enzymes on the virus activity is discussed, and in particular the effect of trypsin is studied experimentally, and shown to be in the main an action on the virus, not necessarily proteolytic. The inactivating effects of normal serum and of silver nitrate are studied, and found also to be due to action on the virus.

The use of the method described may make possible a direct chemical examination of the virus agent even in the presence of other constituents of the plant juice.

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a

b

c

d

Leaves of plant of *N. glutinosa* inoculated with (*a*) $\frac{1}{2}\,0\,0\,0$, (*b*) $\frac{1}{2}\,0\,0$, (*c*) $\frac{1}{2}\,0$, and (*d*) $\frac{1}{2}$ virus concentrations (Aucuba Mosaic) on half-leaves, and same concentrations with addition of 0·25% $AgNO_3$ on other half-leaves. (Photos. by V. Stansfield.)

(*Facing p.* 506)

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581 : 12

Researches on Plant Respiration

IV—The Relation between the Respiration in Air and in Nitrogen of certain Seeds during Germination. (b) Seeds in which Carbohydrates Constitute the Chief Food Reserve

By William Leach

(*Communicated by W. Stiles, F.R.S.—Received November* 28, 1935)

Introduction

Part III of this series (Leach and Dent, 1934) dealt with experimental data concerning the effect on the respiration rates and respiratory quotients of certain fat-storing germinating seeds when successively surrounded by atmospheres of air, nitrogen, and air. The present paper describes similar data relating to the respiration of germinating seeds in which the predominant food reserves are carbohydrates.

The seeds used in the present investigation, as with those used in the previous one, were of species belonging to genera widely separated from one another phyletically. The seeds were all obtained from Messrs. Sutton & Sons of Reading and the species and varieties used were as follows:—

Fagopyrum esculentum, *Zea mais* (Sutton's Improved Japanese Striped), and *Lathyrus odoratus* (" What Joy ").

The seeds of all three species are similar in the respect that in each case starch forms the chief reserve food material.

The experimental data were all obtained by the katharometer method already described (Stiles and Leach, 1931; Leach, 1932) the experimental seedlings being maintained at a constant temperature of 25° C, and under other conditions identical with those used for *Ricinus*, *Helianthus*, and *Cucurbita*. Respiration rates are expressed as milligrams carbon-dioxide