The properties found by FAGER[11] for the activity of carboxylation associated with his suspension of chloroplasts approach those reported here.

SUMMARY

1. Using *Tetragonia expansa* leaves, a carboxylation enzyme system, carboxydismutase, capable of fixing CO_2 with RuDP to give two molecules of phosphoglyceric acid is found free from other enzymes.

2. The enzymic activity is not sensitive to dialysis with certain conditions. It seems to act as an independent system.

3. The enzymic activity is sensitive to *p*-chloromercuribenzoate; this last inhibitor being reversible by addition of cysteine, suggests the participation of –SH groups in the CO_2 fixation.

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Received June 1st, 1956

BIOLOGICAL EFFECTS OF THE INCORPORATION OF THIOURACIL INTO THE RIBONUCLEIC ACID OF TOBACCO MOSAIC VIRUS

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INTRODUCTION

In our previous work[1], we have shown that thiouracil marked with ^{35}S could be incorporated into the ribonucleic acid of growing tobacco mosaic virus. The chromatographic study of hydrolysates of ribonucleic acid modified in this way indicated that the incorporated thiouracil was probably present in the form of thiouridylic acid. MATTHEWS repeated these experiments with a different method, and at first could not verify our results[2,3]. More recently, however, he confirmed and extended them[4].

This confirmation encourages us to publish in more detail some experiments[5] on the biological effects of the structural modifications undergone by the virus ribonucleic acid when thiouracil is incorporated.

References p. 361.

Since thiouracil is an inhibitor which, at a very low concentration, can completely block the growth of tobacco mosaic virus, we ought to consider it as a chemotherapeutic agent acting by an irreversible alteration of the genetic material, that is, a mechanism which has nothing to do with competitive inhibition at the level of an enzyme controlling one of the stages of synthesis of the virus nucleic acid.

MATERIAL AND TECHNIQUES

The tobacco mosaic virus used in the experiments was provided by Dr. BAWDEN.

Cultures were made on young leaves of *Nicotiana tabacum* var. White Burley, divided into fragments and floated on Vickery's nutrient solution.

Material prepared in this way was incubated in Pyrex dishes covered with glass plates and illuminated by a grille of fluorescent strip-lights. Purification of the virus so produced was carried out by the method of COMMONER, slightly modified[6]. The degree of purity of the solutions used for infections was checked by electrophoresis, examination of the crystallized virus by dark-ground illumination, measurement of ribonucleic acid and of total nitrogen. Measurements of infectivity towards *Nicotiana tabacum* were made by means of solutions whose titre had been checked by determination of their protein nitrogen content. Furthermore it was verified that their content of ribonucleic acid corresponded to the concentration of virus found.

These solutions were applied to batches of leaf fragments taken from plants of the same age. All the batches had the same proportions of fragments of leaves occupying the same position on the plants. Entire batches were taken out at different time intervals. The leaf extract was freed of normal cytoplasmic particles by freezing, thawing and centrifuging at 10,000 r.p.m. The virus was then measured after precipitation with antiserum[7]. The precipitates were washed and the total nitrogen of each was determined. The virus content was estimated by means of a reference curve obtained by precipitation, in the same conditions, of virus of known titre.

Measurements of infectivity towards *Nicotiana glutinosa* were carried out by means of the same initial solutions. Physiologically identical batches of leaves, which had been detached from the plants and incubated on damp cotton-wool in dishes covered with glass plates, were infected.

When the leaves of *Nicotiana tabacum* in which the virus was developing were to be subjected ot the action of thiouracil and uracil, they were quickly washed in distilled water and transferred to a nutrient solution containing these substances. The culture conditions otherwise remained the same.

RESULTS

1. *Properties of virus cultured in the presence of thiouracil compared to those of normal virus*

Two physiologically equivalent batches of *Nicotiana tabacum* leaves were simultaneously infected, placed in a damp chamber for 24 hours, washed in distilled water and then incubated on Vickery solution. The leaves of one of the batches were transferred to Vickery solution containing $7 \cdot 10^{-5}$ *M* thiouracil 48 hours after the infection.

After eight days incubation, the virus formed was purified. The quantities recovered for the same weight of leaves were 73.8 mg in the absence of thiouracil (virus *N*) and 17.8 mg in the presence of the inhibitor (virus *TU*). The ribonucleic acid content of the two preparations was the same. The experimental conditions were identical to those of previous experiments[1,5] with thiouracil marked with ^{35}S, when incorporation of the thiouracil into the ribonucleic acid of the virus was observed without exception.

Stock solutions of 0.5% were prepared from the virus obtained in this way, and their concentration was checked by measurements of protein nitrogen. They were diluted immediately before use.

References p. 361.

(a) Young leaves of *Nicotiana tabacum* (10–12 cm) were divided longitudinally in half, the central vein being eliminated. One of the halves of each leaf was infected with the solution of virus *N*, the other with the solution of virus *TU*, both diluted 3^6 times. Each batch of half-leaves weighed about 30 g. Three days after the infection, the virus was measured immunologically in samples of leaf extract. The results of four independent experiments are given in Table I.

TABLE I

	Protein nitrogen of the precipitates (γ/10 ml)	
	Infection with normal virus (N)	*Infection with virus cultured in the presence of thiouracil (TU)*
1	329	116
2	347	106
3	252	99
4	260	106
	297 (mean)	107 (mean)

TABLE II

Dilution of the infecting solution	*Protein nitrogen of the virus precipitates (γ/10 ml)*
3^6	320
	347
3^7	258
	249
3^8	121
	112

It can be seen that the amount of virus produced from the virus cultured in the presence of thiouracil is 64% less than the amount produced from the normal virus.

At the same time as the preceding experiment, the solution of virus *N* was applied at various concentrations to batches of half-leaves which were physiologically equivalent and had approximately the same surface area. The quantities of virus recovered three days after the infection are shown in Table II.

It can be seen from these data that a reduction of 64% in the quantity of virus produced, such as had been observed in the case of infection by the virus *TU*, could only be obtained by a decrease in concentration of virus *N* of approximately 82%.

When virus has been cultured in presence of thiouracil, therefore, its infectivity seems greatly reduced*.

Two hypotheses would account for the results obtained in this experiment. The first would be to suppose that culture of the virus in the presence of thiouracil had made possible the isolation of a mutant, spontaneous or induced, which would be characterized by a growth rate lower than that of the normal virus. The second hypothesis would be that a known weight of the virus cultured in the presence of thiouracil might contain a smaller quantity of infectious material than the same weight of a normal virus preparation. The following experiment made it possible to choose between these two hypotheses.

(b) Two physiologically equivalent batches of 48 g of young leaves of *Nicotiana tabacum* were infected, one with a solution of the virus *N*, the other with a solution of the virus *TU*, each brought to the same concentration of protein nitrogen. This

* We tried to accentuate further the effect of thiouracil by using it at higher concentrations during the synthesis of the virus *TU*. The results were not encouraging. As the concentration of thiouracil rises, its inhibiting effect on the growth of the virus increases. Virus recovered under these conditions contains only a small proportion of particles synthesized in the presence of thiouracil, and which could therefore be modified. All the particles which were already present when the thiouracil was added, especially the excess particles which had remained on the surface of the leaf, have of course the normal properties, for thiouracil has no action on virus that is not growing.

References p. 361.

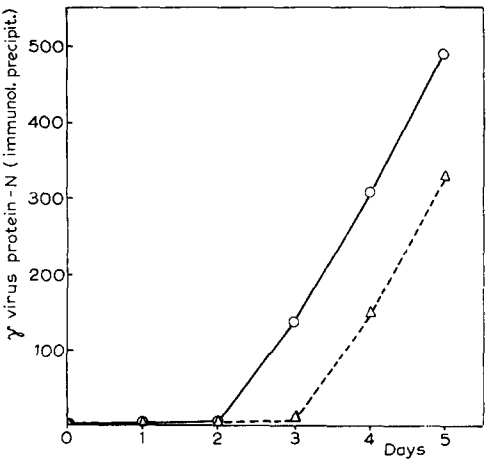

Fig. 1. Quantities of virus obtained from leaves infected at time o with normal virus (*N*) and virus (*TU*) whose nucleic acid contained thiouracil. △---△ virus *TU*; ○—○ virus *N*.

concentration was made low enough for the quantity of virus produced from the virus *N* to decrease when the concentration of the infecting solution was lowered (*cf.* previous experiment). After infection, each leaf was divided into four parts and then incubated. Samples of physiologically equivalent groups of quarter-leaves were taken, each day, and the amount of virus formed was measured.

Fig. 1. shows an example of the results obtained. It can be seen that the two growth curves are parallel, that obtained from the virus *TU* being displaced by 24 hours from that observed for the virus *N*. The growth rate is therefore the same in both cases. The difference is simply in the time necessary before an appreciable increase of the virus can be detected. It follows from this fact that the demonstration of the difference in infectivity of the two solutions is easier the shorter the duration of the experiment, and that a simple comparison between the amounts of virus formed after long periods would not have revealed it.

Since the growth rate of the virus *TU* is the same as that of the virus *N*, the idea of the selection of a slow-growing mutant can be eliminated, and we must conclude that the virus *TU* contains, in an equal weight, a smaller quantity of infectious material than the virus *N*. From this it would appear that either the virus *TU* is made up of a mixture of infectious and non-infectious particles, or that all the particles of the virus *TU* are infectious, but each contains a smaller quantity of infectious material than the particles of virus *N*. The following experiment was designed to obtain information on this point.

(c) Two batches of 40 leaves of *Nicotiana glutinosa*, taken from plants of the same age, and chosen from positions such that the two batches could be considered as physiologically equivalent, were infected, one with the virus *TU*, the other with the virus *N*. The solutions used for the infections were obtained by diluting 3^5 times the stock solutions used in the previous experiments. (It was verified that the number of spots of necrosis which appeared after infection with the virus *N* diluted 3^5 times or more varied rapidly with the dilution.)

The numbers of necrotic spots observed on equivalent surface areas of leaves infected with the virus *N* and the virus *TU* at the same concentration of protein nitrogen were similar, if the probable error of the measurements was taken into account (for example: 5,392 spots in the first case and 5,158 in the second). COMMONER[8] and BAWDEN[9] have previously made the same observations*.

We arrive therefore at the following conclusion. On the one hand, the number of infectious particles in equal weights of the two virus preparations is the same. On the

* MATTHEWS[4] has reported that if tobacco mosaic virus is cultured in the presence of azaguanine there is a reduction in the number of infectious particles per unit weight of virus.

References p. 361.

other hand, the amount of infectious material per unit weight is much smaller in the virus *TU* than in the virus *N*, as the preceding experiments show. Consequently, the mean quantity of infectious material in each particle of the virus *TU* must be less than that contained in one particle of the virus *N*.

Finally, it may be noted that the particles of the virus *TU* and those of the virus *N* penetrate the cells equally easily, and have the same opportunity of starting an infection (expt. c). The fact that the quantities of virus formed from the *TU* particles are smaller than those formed in the same time from the *N* particles (expts. a and b), must be explained therefore by an alteration in properties concerned in the biosynthesis of the virus.

2. *Nature of the modification undergone by the virus cultured in the presence of thiouracil and responsible for its decrease in infectivity towards* Nicotiana tabacum

We shall not reconsider here the demonstration previously given[1] that the nucleic acid of virus cultured in the presence of thiouracil ^{35}S contains a labelled constituent that is chromatographically distinct not only from thiouracil itself, but also from all the constituents of the nucleic acid of the normal virus. We thought that this constituent must be thiouridylic acid, and our hypothesis has recently been confirmed by MATTHEWS[4]. The only new fact available on this subject concerns the extent of the observed incorporation. In our recent experiments, the amount of thiouracil incorporated was from 4 to 18% of the amount of uracil present in the nucleic acid.

In the present work we shall simply offer a piece of evidence in favour of the idea that the incorporation of thiouracil into the ribonucleic acid of the virus is alone responsible for the decrease in its infectivity towards *Nicotiana tabacum*.

Six physiologically equivalent batches of leaves of *Nicotiana tabacum* were simultaneously infected. Three of these batches (left column of Table III) were cultured, the first without addition, the second in the presence of thiuracil ^{35}S, the third in the presence of thiouracil ^{35}S at a 50 times higher molar concentration and non-marked uracil at a molar concentration double that of the thiouracil.

The virus obtained from these first three batches was purified, its ribonucleic acid was isolated by the method of KNIGHT[10], and the quantity of thiouracil incorporated in it was determined.

The three other batches of infected leaves (right column of Table III) received the

TABLE III

	Virus used for the infection	*Thiouracil ^{35}S present in the RNA of the virus used for the infection (c/m/mg).*	*Amount of the virus produced after 6 days (γ protein nitrogen of the precipitate)*
a	Cultured without addition	0	255
b	Cultured in the presence of thiouracil $6 \cdot 10^{-5}$ *M*	920	68
c	Cultured in the presence of thiouracil $3 \cdot 10^{-3}$ *M* and uracil $6 \cdot 10^{-3}$ *M*	114	190

References p. 361.

same additions, except that the thiouracil did not contain ^{35}S. The virus produced was purified in the same way, and was used for the preparation of solutions of the same concentration of protein nitrogen. It was verified that these solutions contained the same concentration of ribonucleic acid. Physiologically equivalent batches of leaves of *Nicotiana tabacum* were infected with these solutions and incubated for 6 days. The amount of virus produced in each was determined immunologically by the usual method.

If the extent of incorporation of thiouracil into the ribonucleic acid of the virus in the different preparations on the one hand, and the infectivity of these same preparations, measured by the amount of virus produced after application on *Nicotiana tabacum* of solutions of the same concentration of each, on the other hand, are considered in parallel, we arrive at the following conclusions (*cf.* Table III).

(1) The addition of uracil to the cultures, in the presence of thiouracil, greatly decreases the amount of thiouracil incorporated into the nucleic acid.

(2) This same addition very strikingly increases the infectivity of the virus, as is shown by the 180% increase in the amount of virus recovered in expt. c (infection with the virus cultured in the presence of thiouracil + uracil) by comparison with the amount recovered in expt. b (infection with the virus cultured in the presence of thiouracil).

This leads therefore to the conclusion that there must be a relationship between the suppression by uracil of the incorporation of thiouracil in the nucleic acid, and the return of the infectivity of the virus to a value close to the normal. This double effect of the uracil is the more striking in that the concentrations of thiouracil, whose effects it largely suppresses (expt. c), are 50 times greater than those which are sufficient to produce these effects (expt. b).

It must be noticed, however, that the nucleic acid does not contain the whole of the radioactivity incorporated by the virus cultured in the presence of thiouracil marked with ^{35}S. The protein part of the virus, separated from the nucleic acid by the method of KNIGHT and treated with 5% perchloric acid in the cold to eliminate the last traces of nucleic acid, still has, in fact, a specific radioactivity equal to 1% of that of the nucleic acid.

The ^{35}S incorporated is probably present in the amino acids synthesized by the host plant from the degradation products of thiouracil. It is certain, however, that the incorporation of ^{35}S into the protein part of the virus takes place by a mechanism that is completely different from that which comes into play when thiouracil is incorporated into the nucleic acid. In fact, in the presence of uracil, the incorporation of ^{35}S into the protein part continues normally, while the incorporation into nucleic acid is greatly inhibited.

What is important to notice in this connection is that the presence of uracil brings the infectivity of the virus cultured in the presence of thiouracil back to a value close to the normal, without however inhibiting the incorporation of ^{35}S into the protein part. This incorporation into the protein has therefore no importance for the infectivity of the virus.

In conclusion, we have established a clear parallelism between the incorporation of thiouracil into the nucleic acid and the reduction of infectivity of the virus. The incorporation of ^{35}S into the protein component has, on the contrary, no effect on the infectivity. Obviously we have no proof that the protein part of the virus cultured in

References p. 361.

the presence of thiouracil has an entirely normal structure. All that we can say is that there is no evidence to the contrary.

3. Influence of thiouracil on the growth of th virus when the inhibitor is kept permanently in contact with the infected leaf

We have so far shown that the culture of virus in the presence of thiouracil leads to the production of particles with reduced biosynthetic power.

In the experiments described, the virus which had been cultured in the presence of thiouracil was tested in the absence of this substance. The observed effect of thiouracil seemed to be the result of its incorporation into the nucleic acid of the virus, and it was irreversible. We shall now examine the effects of thiouracil on the growth of the virus during the first phase of the preceding experiments, that is, when the thiouracil was in contact with the leaves in which the virus was growing. As COMMONER[8], BAWDEN[9] and we ourselves[1], have observed, the inhibiting effect in these conditions is very great, and can lead to the total suppression of virus growth if the thiouracil acts shortly after the infection. This effect of thiouracil could be interpreted in two ways.

Hypothesis (a). It might be a consequence of its incorporation into the nucleic acid of the virus, which could irreversibly modify the structure of this compound, and alter its biosynthetic activity.

Hypothesis (b). It might be the result of a competitive action of the thiouracil or of one of its derivatives, exerted at the level of an enzyme which plays a part in a synthesis starting from uracil or a derivative of uracil.

In principle, it should be possible to decide between these two hypotheses by examining the growth curves of the virus in presence and in absence of thiouracil. However, since the possibility that the two mechanisms act simultaneously cannot be excluded, this sort of approach to the question would set an intricate problem. We chose another method of trying to discern the possible significance of the incorporation of thiouracil into the nucleic acid in the inhibition of growth.

Six physiologically equivalent batches of leaf fragments were infected on the surface, rapidly washed with water, and incubated in the various conditions indicated in Table IV, in which the quantities of virus produced by equal weights of leaves are also shown.

The following information can be derived from the results of such experiments.

(1) Thiouracil, if it is placed in contact with the leaves immediately after their infection completely inhibits virus growth.

(2) This effect of thiouracil is completely suppressed if, immediately after the infection, thiouracil and uracil are added simultaneously (*cf.* expts. a, b and c).

(3) If, on the other hand, thiouracil acts alone for several hours, and the leaves are then transferred to a nutrient solution containing thiouracil and uracil, the growth of the virus is strongly inhibited (compare expts. d, e, f to c). This inhibition is maximal for a six-hour period of action of thiouracil alone.

Let us now consider to what extent these facts can be interpreted by each of the hypotheses envisaged.

(a) Hypothesis of an inhibition by competition between thiouracil and uracil (or their derivatives) at the level of an enzyme playing a part in a synthesis that is important in growth of the virus.

If the inhibition of growth was the result of a mechanism of this kind, the phenom-

References p. 361.

TABLE IV

	Conditions during the incubation	*Protein nitrogen of the virus precipitate (γ)*
a	Nutrient solution without addition: 6 days	1,199
b	Thiouracil 10^{-4} *M*: 6 days	0
c	Thiouracil 10^{-4} *M* + Uracil $2 \cdot 10^{-4}$ *M*: 6 days	1,203
d	Thiouracil 10^{-4} *M*: 2 h Thiouracil 10^{-4} *M* + Uracil $2 \cdot 10^{-4}$ *M*: 6 days	607
e	Thiouracil 10^{-4} *M*: 6 h Thiouracil 10^{-4} *M* + Uracil $2 \cdot 10^{-4}$ *M*: 6 days	329
f	Thiouracil 10^{-4} *M*: 24 h Thiouracil 10^{-4} *M* + Uracil $2 \cdot 10^{-4}$ *M*: 6 days	428
g	Thiouracil 10^{-4} *M*: 48 h Thiouracil 10^{-4} *M* + Uracil $2 \cdot 10^{-4}$ *M*: 6 days	390

enon would be reversible by uracil. The presence of thiouracil alone during an initial period of the experiment would simply bring about a delay in the growth of the virus, and growth would then resume its normal rate in the presence of uracil. The amounts of virus recovered six days after addition of the uracil in expts. c and e, for example, would then be equal, and the inhibition of 72% observed in e would be inexplicable.

(b) Hypothesis that the inhibition of growth is the result of incorporation of thiouracil into the virus nucleic acid.

In accordance with this hypothesis, we should consider that during the period in which the thiouracil acts alone it is incorporated into the nucleic acid of the newly formed virus and prevents it from playing its part in the synthesis of new virus. When the uracil is later added, this incorporation stops; but the nucleic acid which has already been structurally altered cannot recover its normal biosynthetic functions. Growth of the virus can therefore only take place under the influence of that part of the nucleic acid that has remained intact, and so will be reduced in amount. On the other hand, when uracil is added at the same time as the thiouracil, there is no incorporation of the thiouracil, and growth is brought about by means of the whole of the nucleic acid introduced into the cells by the infection. Growth will therefore be more rapid in the second case (expt. c) than in the first (expt. e).

The second hypothesis therefore accounts for the observed facts while the first does not.

The experiments reported above suggest two further considerations.

An irreversible reduction by thiouracil of the quantity of virus formed, of the order of 72%, such as we observe in case e (Table IV) corresponds to a reduction of 99% of the concentration of virus in the infecting solution (Table II). If we consider, as suggested in the above discussion, that the 72% reduction observed must be attributed to the incorporation of thiouracil into the nucleic acid, we are led to suppose that this incorporation alone can have massive effects on the quantity of infectious material present in the virus, and is sufficient to explain the drastic action of thiouracil on its growth.

References p. 361.

It should furthermore be noticed that when thiouracil had acted alone for periods of 6 hours, 24 hours and 48 hours, the amount of virus formed after 6 days in the presence of uracil was the same. It appears therefore that the same amount of infectious material was present in the three cases at the moment that the uracil was added. The simplest explanation of this fact seems to be that the thiouracil, on being incorporated into the newly formed nucleic acid molecules, had inactivated all of them irreversibly and that growth in the presence of uracil took place from molecules introduced during infection, the same quantity in all three cases. Here again it appears that thiouracil can exert a powerful action simply by its incorporation.

BAWDEN[11] has reported that thiouracil inhibits the multiplication of various viruses cultured on certain plants, but is without action if the host belongs to another species. If our hypothesis is correct, incorporation of thiouracil into the nucleic acid of the virus should be observed in systems of the first kind, but not in those of the second. Such a difference would not be surprising, and could be explained by greater or less specificity of the enzymes which take part in the synthesis of nucleic acid, according to the plant species which provides them.

According to a hypothesis put forward by KALCKAR[12], metabolic analogues of purines and pyrimidines could act as traps for ribose, by fixing this substance in the form of abnormal nucleosides that could not be used by the cell. Such a hypothesis would appear to be inapplicable to the case examined in this work. The thiouracil is, most active as inhibitor when it is applied in the first hours after the infection, that is, at a time when the quantities of ribose used in building the virus are smallest. It becomes in contrast only slightly active at the same concentrations when the virus has become abundant and the quantities of ribose used at each duplication are much greater.

DISCUSSION AND CONCLUSIONS

The significance of the experimental facts described in this work has been discussed immediately after the description of each. Here we shall only sum up the general interpretation at which we have arrived. For this purpose, we shall use the term "amount of infectious material" contained in a virus particle, for the component of this particle which plays an effective part in the biosynthetic processes leading to the formation of new virus particles inside the host cell. Our interpretation can then be expressed by saying that the culture of tobacco mosaic virus in the presence of thiouracil decreases the amount of infectious material contained in each particle without modifying either the amount of protein or the amount of nucleic acid which it contains. We must therefore suppose that the presence of thiouracil has entailed a structural modification of a variable part of the molecules of proteins or nucleic acid normally playing this biosynthetic role. On the other hand we have established that the decrease in the amount of infectious material contained in a particle does not necessarily suppress its infectious character. Consequently the "infectious material" in a single particle must contain several collections of specific structures, each capable of assuring the transmission of the hereditary characters of this particle.

Since our experiments showed that the decrease in the amount of infectious material in a virus particle was accompanied by incorporation of thiouracil into the nucleic acid component, probably in the form of thiouridylic acid, we consider it possible to

References p. 361.

attribute to this incorporation the inactivation of a variable part of the "infectious material" of the particle. This hypothesis is based upon the idea, evidence for which was presented in a previous piece of work[13], that the ribonucleic acid of the plant virus might play a biosynthetic part analogous to that which has been generally attributed to the desoxyribonucleic acid of phages since the well-known experiments of HERSHEY on the multiplication mechanism of the latter*.

The preceding conclusions are the only ones which we can draw from the facts known at present. The interpretation of our experiments could only be pushed further if the structure of the axial rod of ribonucleic acid of the particles of tobacco mosaic virus were elucidated, and if our knowledge of the genetics of this virus were more developed. We shall only indicate here in an appendix what seems to us a possible line of research.

APPENDIX

As remarked above, we have been led to suppose the existence, in each virus particle of several collections of specific structures, each capable of assuring the transmission of the hereditary characters of this particle. Let us call each of these collections a genome. If a molecule of virus ribonucleic acid be considered to have a molecular weight of 290,000 or 250,000[15] there would be room for about eight of these molecules in a virus particle. Each of these molecules, if completely unwound, would have about the same length as the particle[15], and could represent a genome. The incorporation of thiouracil into the nucleic acid could inactivate a variable number of these genomes, and if only one of them kept its normal structure it would be sufficient for the virus to remain infectious. Finally the hypothesis that there exist in each particle eight genomes, each of which could be separately inactivated by the thiouracil, would account for the experimental facts described in this work. Calculation shows in fact that, for a number of genomes of this order, a decrease of 80% in the amount of infectious material (experiment I) corresponding to a known weight of virus, that is, a decrease of 80% in the number of active genomes contained in them, would entail a decrease of only 20% in the number of infectious particles, that is, a decrease of the same order of magnitude as the experimental error made in the determination of this number.

Finally it might be interesting to examine the consequences, of a quantitative type, of a hypothesis of this kind**.

Let us suppose as above, that each virus particle contains eight genomes, corresponding to as many molecules of ribonucleic acid of molecular weight 290,000. Let us assume, furthermore, that of the 200 uridylic nucleotides which a genome contains, n ($1 \geqslant n \geqslant 200$) occupy a position such that if one of them is replaced by a thiouridylic nucleotide, the genome in which this replacement has occurred is inactivated.

The probability that one uracil is not replaced by one thiouracil being a, the probability A that, of n molecules of uracil none will be replaced by thiouracil is a^n. Experimentally a is measured by the proportion of the uracil molecules that are not replaced by thiouracil and A by the proportion of the genomes that remain active.

Let us consider by way of example the case in which the replacement of 10% of

* GIERER AND SCHRAMM[14] have recently shown that it is possible to infect tobacco leaves with RNA from TMV in the total absence of the protein component of the virus.

** I should like to thank Dr. R. THOMAS for this suggestion.

References p. 361.

the uracil groups by thiouracil would result in a decrease of 82% of the total number of active genomes contained in a known weight of virus. Here $a = 0.9$; $A = 0.20$; therefore $n = 16$.

This recalls the observation of HERSHEY *et al.*[16] on the effects of the decay of ^{32}P atoms incorporated in the DNA of phage, which could be interpreted by saying that there is 1 chance out of 10 that the alteration by decay of a single nucleotide in the phage particle renders it inactive.

SUMMARY

1. Tobacco mosaic virus, grown in the presence of thiouracil contains per unit weight a reduced quantity of material susceptible of forming new virus after infecting *Nicotiana tabacum*. This reduction in the quantity of infectious material appears to be related to the incorporation of thiouracil into the virus nucleic acid. If uracil is added to the culture, at the same time as thiouracil, the incorporation of the latter in the nucleic acid is considerably diminished and the quantity of infectious material contained in the virus is normal. The protein part of the virus incorporates a small quantity of the S from the thiouracil. This incorporation is without effect on virus infectivity.

2. On the other hand the incorporation of thiouracil in virus nucleic acid does not modify appreciably the number of particles per unit weight of virus that are capable of producing a necrotic lesion in *Nicotiana glutinosa*. This incorporation is thus capable of diminishing the average quantity of infectious material contained in each particle without necessarily suppressing the infectivity of the whole particle. Each virus particle appears thus to contain several series of specific structures responsible for the transmission of the genetic characters of the virus.

3. When virus is cultivated in the presence of thiouracil, uracil being added after a few hours, the growth of the virus appears strongly inhibited when compared to a control where thiouracil and uracil had been added simultaneously at the beginning of the culture. The inhibitory effect of the incorporation of thiouracil into nucleic acid is sufficiently important to be considered as the major cause of the inhibitory action of thiouracil on the growth of the virus. Thiouracil does not appear to act appreciably as a competitive inhibitor of a synthetic mechanism indispensable for virus growth.

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Received May 31st, 1956