Thus, although admittedly too high, it is apparent that the magnitude of the gold crystals does give some indication of the size of the spaces present in the fibre. The results given in Table I are also consistent with the view that non-cellulosic constituents are located within the larger intermicellar regions of the fibre although the more recent data of SEN AND WOODS[3] makes it probable that lignin is present at the edges of the finest capillaries of the cell wall, probably in association with paracrystalline cellulose surrounding the micelles[4].

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A PRELIMINARY STUDY OF THE INCORPORATION IN GROWING TURNIP YELLOW MOSAIC VIRUS AND ITS RELATED NON-INFECTIVE ANTIGEN OF LABELLED AMINO ACIDS

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The presence in plants infected with tobacco mosaic virus of a non-infective RNA-free protein having a much lower sedimentation rate than the virus, but crystallizing in needles like it and having very similar immunological properties, has recently been reported[1,2,3,4]. Similar observations had already been described by MARKHAM[5] in the case of turnip yellow mosaic virus (TYMV) where the virus and its antigen have however more similar sedimentation constants. Our lack of knowledge about the signification of these non-infective proteins and especially about their possible role in virus multiplication has prompted us to approach such problems with the aid of radioactive tracers, a method which does not seem to have been used so far for this purpose.

When chinese cabbage plants are infected with TYMV, a massive infection appears only in the new leaves which grow after infection. The virus, the RNA-free antigen and the normal proteins are synthesized simultaneously and form an actively growing system where the proportions of these constituents remain approximately constant. Such conditions are sufficiently simple to give us some indications of the relations that might exist between the virus and its associated antigen on the basis of measurements of the rate of incorporation of labelled amino acids in these two components. In the experiments to be reported here we always used plants bearing only these new leaves, the others having previously been sectioned.

Plants infected 2 or 3 weeks beforehand, cultured in identical conditions and as similar in size as possible, are placed, in the presence of $^{14}CO_2$, in a glass cage strongly illuminated by fluorescent tubes. After 5 or 21 hours, the virus and non-infective antigen are separated from the normal proteins of the extract by alcohol fractionation, followed by several crystallizations in the presence of ammonium sulfate[5]. A 6–8% solution of the crystals is then fractionated by centrifugation, according to the technique of MARKHAM[5,6], into the two constituents described by this author: the infective virus containing 28% RNA and a protein immunologically similar but containing no RNA or only traces. An electrophoretic study, in the PERKINS-ELMER apparatus, shows that the two fractions which have been isolated contain no observable amounts of normal proteins. Both give very similar precipitation curves with the serum of a rabbit immunised against purified virus (determination of the nitrogen of the precipitate as a function of the nitrogen of the antigen, added to a constant quantity of antiserum).

After precipitation with trichloracetic acid, washing of the precipitate and extraction with alcohol-ether, the RNA is eliminated by cold 10% perchloric acid. Specific radioactivities of the dry powders finally obtained are determined on equal weights of material with a thin window counter, taking care to avoid geometrical errors. The results are shown in Table I. The differences observed between the specific radioactivities of the virus and of its RNA-free antigen have been confirmed

by determinations of the radioactivity of the CO_2 obtained by the action of a specific glutamic acid decarboxylase on protein hydrolysates, and collected as the Ba salt.

If we admit that both the proteins studied have been synthesized from a common pool of amino acids marked by ^{14}C through photosynthesis, the finding that the specific radioactivity of the RNA-free antigen is always higher than the specific radioactivity of the virus excludes the hypothesis that it is a degradation product of the virus by loss of RNA. If the hypothesis of such a degradation were true, we would expect the virus to have a higher specific radioactivity than the antigen, being its precursor.

The hypothesis of a degradation of the virus being excluded, we could imagine that the protein and nucleic acid fractions of the virus are synthesized independently and united later during the formation of the definitive virus particles. The RNA-free antigen could then be the protein portion of the not yet completed virus. In this case, we should expect:

1. that the specific radioactivity of the RNA-free antigen would be higher than that of the definitive virus particles, at least for short time incorporations.

2. that the ratio existing between the specific radioactivity of the antigen and that of the virus diminishes as the duration of incorporation increases.

All the measurements done so far fulfil these conditions. We should however insist on the fact that, if these data agree with the hypothesis that the RNA-free antigen is the protein portion of the virus in formation, they cannot alone be taken for a demonstration of this idea. However, a strong argument in favor of the interpretation we suggest is that it agrees with the image of the mechanism of virus synthesis which progressively arises from all that the study of bacteriophages has brought to light.

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TABLE I

	Technique for the measurement of the specific radioactivity	*Duration of incorporation*	*Spec. radio-act. of the virus*	*Spec. radio-act. of the RNA-free antigen*
1	determ. on total prot.	5	18	32
2	determ. on total prot.	21	170	241
3	determ. on total prot.	5	18	51
4	determ. on total prot.	21	89	149
5	determ. on CO_2 from glut. acid decarboxylation	5	10	37
6	determ. on CO_2 from glut. acid decarboxylation	21	61	82

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