The role of the soluble antigens in the multiplication of the tobacco mosaic virus

In the course of the last few years many workers have called attention to the existence, in plants infected with a virus, of protein constituents of the same immunological specificity as the virus, but non-infectious and lacking ribonucleic acid[1–5]. The significance of these constituents is not yet understood. Preliminary work[6] on the turnip yellow mosaic virus has, however, eliminated the possibility that the non-infectious protein constituent might be a degradation product of the virus. Indeed, the incorporation of "labelled" amino-acids into the non-infectious constituent is more rapid than into virus. This is in spite of the fact that both virus and non-infectious constituents accumulate in the same leaves at the same rate, presumably from the same amino-acid pool.

The same problem was approached by DELWICHE *et al.*[7], who used the tobacco mosaic virus, and introduced ^{15}N into the infected leaves in the form of ammonium chloride. This work has remained without a definite conclusion, the authors having reported a more rapid incorporation in the constituents without RNA than in the virus, but also that at the time of the experiment the rate of increase of these constituents was greater than that of the virus, which alone would explain their results.

We have carried out a study of the same kind with the tobacco mosaic virus and the protein constituents without RNA (soluble antigens) which accompany it. The results were made more striking by greatly reducing the time of incorporation of the amino-acids, and by using $^{14}CO_2$ as precursor, the plants being intensely illuminated. The tobacco leaves, infected 3 days before, in which the virus was actively growing, were brought into contact with the $^{14}CO_2$ for 30, 60 or 240 minutes. The virus was then isolated by centrifugation and purified by COMMONER's technique. The soluble antigens (which were present in too small a quantity to be isolated by any other method) were precipitated by a specific anti-serum mixed with the extract of the leaves, freed from virus particles by centrifugation. Finally the normal proteins, which do not sediment in 45 min at 90,000 *g* are also collected. The specific radioactivities of the virus, of the soluble antigens and of the normal proteins are shown in Table I. The specific radioactivity of the soluble antigens is calculated from that of the immunological precipitate. In order to do this, the content of soluble antigen in this precipitate is determined in a parallel experiment carried out by means of purified soluble antigens marked with ^{131}I and precipitated under the same conditions.

TABLE I

	Specific radioactivities after:		
	30 min incorp.	*60 min incorp.*	*240 min incorp.*
Soluble antigens	270	980	3,280
Virus	1.5	12	250
Normal proteins	4	16	56

Examination of the results leads to the following observations. Two of the fractions, the soluble antigens and the normal proteins, do not increase during the experiment. However, the soluble antigens acquire in 30 min a specific radioactivity nearly 70 times as great as that of the normal proteins. This fact alone indicates that they must play a very special part in the cell. If their concentration remains constant while they are being synthesised with great rapidity, it is to be predicted that they are being as rapidly transformed into a different protein, which itself accumulates in the cell. The virus is the only protein in these cells which has this characteristic. We are thus led to put forward the hypothesis that the soluble antigens are the precursors of the protein part of the virus. The way in which the specific radioactivity of the virus increases with time is in perfect agreement with such a hypothesis. In fact it increases more rapidly than that of the normal protein while remaining very much less than the specific radioactivity of the soluble antigens. Further, the ratio between the specific radioactivity of the soluble antigens and that of the virus decreases with time, as one would expect if the completed virus represents a kind of reservoir into which flows a continuous stream of soluble antigens. Finally, the hypothesis that soluble antigens are the exclusive precursors of the protein part of the virus can be quantitatively verified by means of available data. Knowing the rate of increase of the virus, the mean specific radioactivity of the soluble antigens, and the ratio between the quantity of soluble antigens and the quantity of virus, one can calculate the specific radioactivity which would be acquired by the virus after a given time according to the hypothesis presented. The specific radioactivities calculated in this way correspond in a very satisfactory manner with the measured radioactivities.

If the protein part of the virus appears initially in the cells in the form of soluble antigens, with low molecular weight and without ribonucleic acid, the latter must be synthesised independently. One is thus led to imagine that the maturation of the virus, that is the acquisition of its infectious properties, must result from the final union of the ribonucleic acid thus synthesised with the soluble antigens to form a huge elongated particle, which is the only state of the constituents of the virus in which infectivity is displayed.

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A rapid colorimetric distinction between glucosamine and galactosamine

Published methods for distinguishing between glucosamine and galactosamine in small quantities depend on their separation, or the separation of substances derived from them, by paper or column chromatography[1-4]. Borate depresses colour formation in the well known colorimetric estimation of ELSON AND MORGAN[5,6]. Under the conditions to be described this depression is of the order of 50% for galactosamine and 75% for glucosamine. If therefore in the routine determination of amino sugars by the ELSON AND MORGAN method a second set of samples of double the amino sugar content is heated with borate in addition to the usual reagents the sugars can be distinguished (using double the volume of amino sugar sample with borate avoids undue spread of colour intensity). If galactosamine alone is present the colour intensity in the two sets after development will be about the same, if glucosamine alone is present the intensity in the borate set will be about half that in the first set. If mixtures are present intermediate values will be obtained.

Aliquots containing 2–8 μg amino sugar N are made to a final volume of 1 or 2 ml in tubes calibrated at 10 ml and matched for use in a photoelectric colorimeter. A second set is prepared in which the volume of each sample is double that in the first and the final volume is the same as in the first set. Two sets of standards of glucosamine and galactosamine, the second containing double the amounts in the first are also set up together with two blanks. 1 ml of borate solution (3.2 g $Na_2B_4O_7 \cdot 10H_2O$ in 100 ml water) is added to the samples and standards of doubled concentration and to a blank. 1 ml of water is added to the other set. To all tubes 1 ml of acetylacetone solution (1 vol. redistilled acetylacetone in 25 vols. 1.5 *N* Na_2CO_3) is added and the contents of the tubes well mixed. The tubes are closed with glass balls, heated for 7 min in a boiling water bath and cooled in cold water. Ethanol is added to the tubes to bring the total volume to 10 ml and is followed by the addition of 1 ml of Ehrlich reagent (1.6 g purified *p*-dimethylaminobenzaldehyde in 30 ml conc. HCl and 30 ml ethanol). The contents of the tubes are mixed by thorough shaking in an inverted position. After development (30–45 mins) the colour intensities are read in a suitable colorimeter using a filter with a maximum transmission in the region of 520–540 mμ. There should be little difference between the values for the two sets of galactosamine standards while the glucosamine standards that have been heated in the presence of borate should give readings about half of those in the control set. The effect of borate is rather variable and it is therefore advisable to include both sets of standards. In terms of colour produced/μg N the colour intensity in the presence of borate has been found to vary between 24 and 30% of the control (glucosamine) and between 50 and 65% of the control (galactosamine). The composition of mixtures of the two amino sugars can be estimated with an accuracy of about 5%. If values for the colour produced in the presence of borate as a % of that produced in its absence are calculated for known mixtures it will be found that the results, if plotted against the composition, will fall on a straight line joining the values for glucosamine and galactosamine. The method gave satisfactory results when applied to hydrolysates of known mixtures of chitin and chondroitin sulphate.

The addition of borate has little or no effect on the form of the absorption curve of the coloured complex finally produced in the reaction. The final pH of the solutions before heating is also unaffected