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INFLUENCE OF THIOURACIL INCORPORATION IN THE RIBONUCLEIC ACID MOIETY OF TOBACCO MOSAIC VIRUS ON ITS MULTIPLICATION

by

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We have recently shown[1] that the ribonucleic acid of tobacco mosaic virus can incorporate i mportant amounts of thiouracil in the form of an unidentified component, chromatographically different from thiouracil and from the normal constituents of ribonucleic acid. At the same time, MATTHEWS[2] gave evidence for the similar incorporation of 8-azaguanine in the ribonucleic acid of the same virus.

Tobacco mosaic virus[3], as well as turnip yellow mosaic virus[4], is not infective if it does not contain ribonucleic acid; this suggests that the ribonucleic acid of plant virus plays an essential role in their multiplication, as does desoxyribonucleic acid in the case of bacteriophage[5]. A change in the composition of ribonucleic acid obtained by incorporation of thiouracil could be expected to have a marked influence on the multiplication of tobacco mosaic virus; the following experiment was thus attempted.

Ten tobacco leaves are used for each experiment. Each leaf is divided longitudinally along its principal vein in two halves. One half is infected with a solution of virus cultured *in vitro* according to the technique of COMMONER[6]. The other half is infected with a solution of virus of the same concentration, cultured simultaneously and purified by the same method; but in this virus 10–12% of the uracil present in the ribonucleic acid has been replaced by thiouracil. The half leaves thus infected are washed and placed for 3 to 8 days on the surface of VICKERY's nutrient medium. The newly formed virus is extracted quantitatively and titrated either by addition of an antiserum under conditions of maximum precipitation, or by the method described by COMMONER[7]. The two methods give comparable results. Those obtained by the immunological method are described in the following table. The amount of precipitated protein is always smaller in the case of leaves infected with thiouracil-containing virus. The fall in concentration of infective particles, which was to be expected from the presence of thiouracil, was determined by comparing these results with those of parallel experiments in which solutions of normal virus were applied, at various dilutions, on physiologically comparable leaves and in which the newly formed virus was titrated as above. An example of the results is given in Fig. 1. As can be seen in the last column of Table I, the fall in concentration amounted to 28–92%.

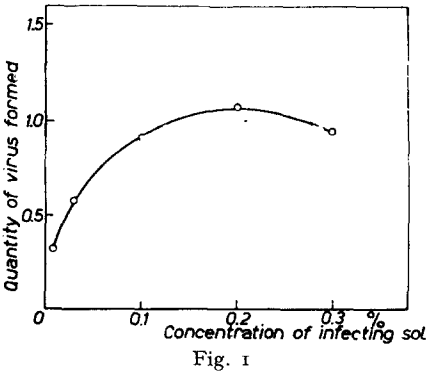

Fig. 1

It should be possible to demonstrate such an important drop in the number of infective particles by applying the virus solution on leaves of *Nicotiana glutinosa* and counting the number of lesions produced. However COMMONER[7] has stated that virus cultivated in the presence of thiouracil retains a normal infectivity when submitted to this test. Thus it appears that a particle of virus which has incorporated thiouracil in its ribonucleic acid remains infectious. Our results show, on the other hand, that the amount of virus it can produce in a short time is considerably reduced. This apparent

contradiction can be easily explained if we admit (SCHRAMM[8]) that the virus particle is constituted of a great number of smaller units, genetically identical, and which multiply independently in the host cell. We may suppose that the thiouracil incorporated in our experiments is not in sufficient amount to inhibit the multiplication of all the elementary units of each particle. Each virus particle will thus remain infective, but the proportion of its elementary units capable of duplication in the host is reduced.

We are well aware of the tentative character of this interpretation and have presented it in order to show that there is no necessary contradiction between COMMONER's results[7] and our own.

TABLE I

Exp. No.	*Duration of virus growth (days)*	*Concentration of infective solut. %*	*Protein N ppt. by antiserum (γ Protein N p. mg Prot. N of leaves)*		*% Drop in the amount of newly formed virus*	*% Drop of concentration of normal virus sol. producing same effect*
			Normal virus	*Thiouracil-containing virus*		
12–10	3	0.1	51	46	9.8	28
		0.3	76	40	47.5	92
5–10	3	0.3	89	68	23	80
29–8	3	0.46	71	53	25	65
23–6	4	1	6	3.7	38.4	68
	7	1	42	32	24	—
	8	1	40	33	17	—

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EFFECT OF TAURO-CHOLIC ACID ON THE pH/ACTIVITY CURVE OF RAT PANCREATIC LIPASE

by

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The bile acids have generally been assumed to activate the pancreatic lipase[1,2,3,4]. These accelerating effects of bile acids on pancreatic lipolysis have mostly been studied in the pH range 8.5–8.9, but at an acid pH an activating effect has also been found[5]. Any detailed study of the effect of pH on the pancreatic lipolysis in the presence of bile acids does not, however, seem to have been undertaken.

The effect of different concentrations of synthetic tauro-cholic acid, at different pH values, on the rate of hydrolysis of olive oil by rat pancreatic lipase has been studied. The results of this investigation are summarized in Fig. 1.

In the absence of tauro-cholic acid the pH/activity curve for the rat pancreatic lipase shows an optimum at pH about 8. With increasing concentrations of tauro-cholic acid the pH optimum is changed to about 7.2 at 0.05 %, pH 6.7 at 0.1 % and about pH 6 at 0.2 % tauro-cholic acid. With increasing concentrations of tauro-cholic acid a second pH optimum also appears at a pH of about 9.