Changes in the contents of RNA and protein as well as cell ultrastructure as influenced by the infection with potato virus X

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INTRODUCTION

Virus reproduction proceeds at the expense of cell materials. It is accompanied by a complete change in both biochemical and physiological processes of the infected cell which lead finally to the biosynthesis of viral protein and viral RNA. At the same time there appear pathological changes in the cell structure.

There is an ample literature concerning the physiology and biochemistry of plant virus infection and their cytopathology. However the majority of those publications deals with rather narrow, separate problems and different virus diseases. In the present work the author tried to investigate the pathological process caused by one of the most wide spread on the globe plant virus — the potato virus X, at different aspects. As the defence reaction of the infected plant may depend both on the infected plant species and on the virulence of the virus strain, experiments were carried out on several different plants, using three different virus strains. The obtained results were published successively in scientific literature, quoted in references. The present paper deals with those results which concern still poorly investigated metabolism of proteins and nucleic acids as well as the ultrastructural cytopathogenic changes. In such aspect the obtained experimental data are published for the first time.

MATERIAL AND METHODS

As host plants following plant species were used: *Datura Stramonium*, *Solanum demissum* and several potato varieties of *S. Tuberosum*. Those were: the early variety Prijekulski early, which is one of the most widely spread in the U.S.S.R., the variety Kamieraz I a medium late variety, which gives characteristic symptoms of virus X infection, and the variety Majestic — used as an international test plant for this virus.

During the winter the plants were cultivated in the greenhouse in pots with garden soil, and in summer they were transferred to a vegetation house, one wall of which consisted of a nylon net in order to promote aeration and to prevent it against insects.

All the experimental plants: *Datura*, wild potatoes and also the cultivated potato varieties (Majestic and Prijekulski early) were grown out of seeds, and in the 4 leaves stage they were inoculated with virus suspension, mixed with karborundum. 20 plants of each species were inoculated in each experimental set. The control plants were inoculated at the same time with water. In the first experimental series the plants of potato varieties: Prijekulski early and Kamieraz I were grown from tubers, the tubers of virus infected plants being derived from plants infected 3 years before. The progress of infection was tested one day after artificial inoculation or one day after sprouting of the diseased tubers. For experiments three different strains of the potato virus X were used, isolated in three different climatic regions of the U.S.S.R: X_s — a severe strain derived from the semi-arid region of Kazachstan, X_k — medium severe, from Kijev, and X_r — a mild strain with diffuse, chlorotic symptoms — from south-eastern part of the R.F.S.R.R. [1]. All those strains contain both common and strain specific antigen groups [2]. Their properties do not change when maintained on *Datura* and potatoes in laboratory conditions [3].

The content of total RNA and DNA in leaves of *Datura* and several potato varieties was determined in different stages of viral infection. In the first experimental series different host plants were infected with the same strain of the potato virus X_k. In the second series, one potato variety and *Datura* were inoculated with three strains of virus X separately. The RNA content was determined spectrophotometrically using a method specially adapted to plant material [4]. This method was based on the asumption, that the RNA absorbancy values at 260 mμ are usually increased by the presence of certain impurities and therefore it seems essential to substract from these values their absorption coefficient. A second wavelength in the UV absorption spectrum of the mentioned impurities was found, at which the optical density was equal to that measured at 260 mμ. This second "semi wave" was in the case of *Datura* and potato nucleoproteides equal to 282 mμ. The quantitative estimation of RNA content in the preparations was done according to the formula of Caniew and Markov [5]:

$$\text{RNA}\,(\text{mg\%P}) = K\frac{(D_{260}-D_{282})\times Y}{W\times 1}$$

in which:

Y — the volume of extract,
W — the weight of the RNA preparation,
1 — thickness of the scanned liquid layer,
K — coefficient estimated empirically for 5.69 [4].

Leaf samples for analyses were taken during the multiplication phase of virus infection. Every sample represented an average compiled according to the following scheme; for the first analysis the first leaf was taken from the first plant, the second — from the second plant etc. At the subsequent sampling from the first plant was taken the second leaf, from the second plant — the third leaf etc.

The plant material was washed free from acid-water-alcool-and ether soluble compounds, until a completely white pulver was obtained [6]. This dried up pulver

was stored in the refrigerator and used for the determination of both nucleic acid, protein and amino acid contents.

Recently, together with the colleague Mielniczenko I analysed the same samples by the method of Fritz and Röttger [6] for their nucleotides content. This enabled us to corelate the observed variations in RNA content with one of the qualitative characteristics. Proteins were determined by the micro-Kjeldahl method, and the aminoacids — by paper chromatography [7].

Virus multiplication in the infected plants was followed by means of serological titration [8].

Simultaneously the analysed samples were examined in the electron microscope. For this purpose from the leaf blade, long, thin pieces of tissue were dissected which exhibited traces of primary virus infection (in those areas tiny, blistering spots appeared 2 days after inoculation, which subsequently became necrotic). The dissected pieces were then fixed in glutaraldehyde for 2 hrs, followed by 1% solution of OsO_4 for another 2 hrs. The preparations were still contrasted afterwards with lead acetate and mounted finally in the mixture of akrylate (AKR-7) and n-butylmetacrylate. The ultrasectioning was done by means of ultramicrotome UMD-5. The sections were examined in the electron microscope JEM-7.

BIOCHEMICAL CHANGES IN LEAVES OF POTATO AND *DATURA*, INFECTED WITH THE POTATO VIRUS X

A. RNA AND DNA CONTENTS

In all examined host-plants some commune changes regarding the influence of virus infection on the nucleic acid content, were stated (Figs 1-5). On the 5th day after infection there is a decrease in the total RNA content but later on its concentration increases 1.5-2 times above the control level and is maintained starting from this time at a higher level during the whole period of plant growth. Only at the very end of the vegetation period the RNA content of the diseased plants decreases below that of the healthy control ones (Figs 2-5). In those experiments in which RNA was investigated in potato plants grown for three consecutive years from virus infected tubers, the RNA content of the diseased plants was always higher in comparison with the control in case of the variety Kamieraz I, while it decreased below that level at the very end of the vegetation in case of the early variety Prijekulski Carly (Figs 3-4). Thus in this second case the same tendency was observed as in plants cultivated from seeds with only one year infection [9]. Variations in the content of DNA were not so clear. However a common tendency of increase of the DNA content in the diseased plants could be stated (Figs 1-5), the differences being nevertheless so small, that their significance is doubtful. If the relative RNA concentrations are recalculated on the basis of the DNA contents, a clear tendency of RNA decrease could be stated at the end of the vegetation period (Figs 1-3).

When putting the above data on a graph (Fig. 6 A and B), one obtains in certain

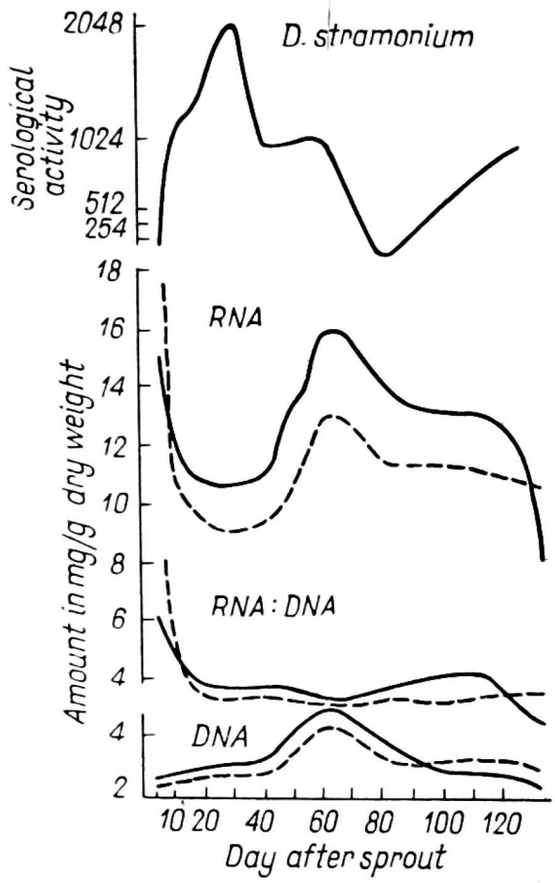

Fig. 1. RNA and DNA content in leaves of *Datura stramonium* during multiplication of the X_k strain of the potato virus X. Explanations: The curve at the top denotes virus titer as estimated serologically. The curves below represent RNA, RNA/DNA and DNA contents respectively. The solid line represents virus infected plants, the dotted line — healthy plants.

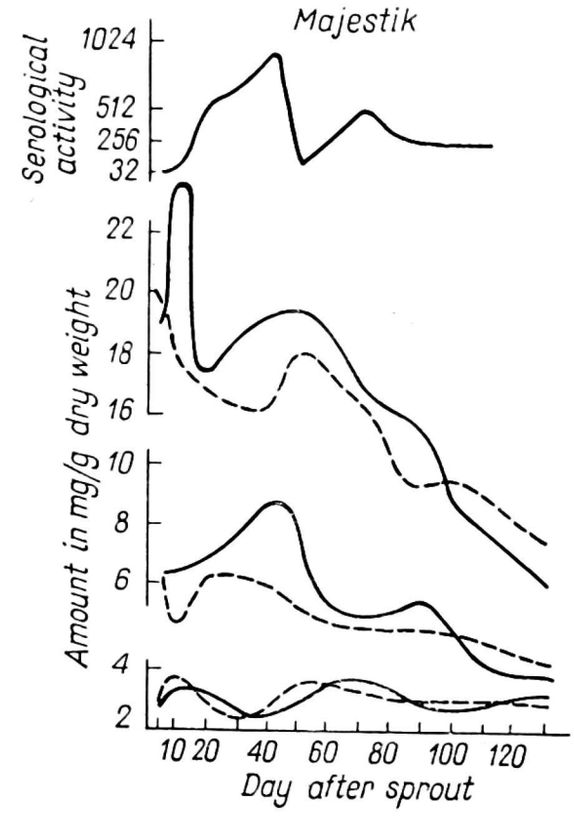

Fig. 2. Changes in nucleic acid contents in leaves of potato variety Majestic at different stages of infection with the potato virus X strain X_k. Explanations as in Fig. 1 and in text.

cases, especially in 5-10 days after infection, a characteristic stepwise decrease of RNA content in virus infected plants, the scope of which depends on the virulence of particular strains. This decrease was most pronounced five days after inoculation of seedlings of the potato variety Prijekulski early and *Datura* with the mild strain (X_r) of the potato virus X (Fig. 6), whereas it was almost invisible after inoculation of the same plants with the semi-virulent X_k strain, and disappeared completely when the virulent X_s strain was used in the experiment.

B. NUCLEOTIDE CONTENT

Analyses of the nucleotide contents of total RNA were carried out with the view to explain the correlation between the observed decrease in total RNA in the 5 day after infection as well as its increase at the 17th day, with the variations in contents of separate nucleotides. Analyses were carried out on *Datura* plants.

It was shown, that apart of the strain X_s at the 5th day after infection, other strains did not cause any significant change in GMP and UMP contents of the infected plants (Table 1). The decrease in total RNA observed after infection with the X_r strain at the 5th day, was most obviously correlated with a simultaneous decrease in AMP, which was maintained below the control level still after 17 days

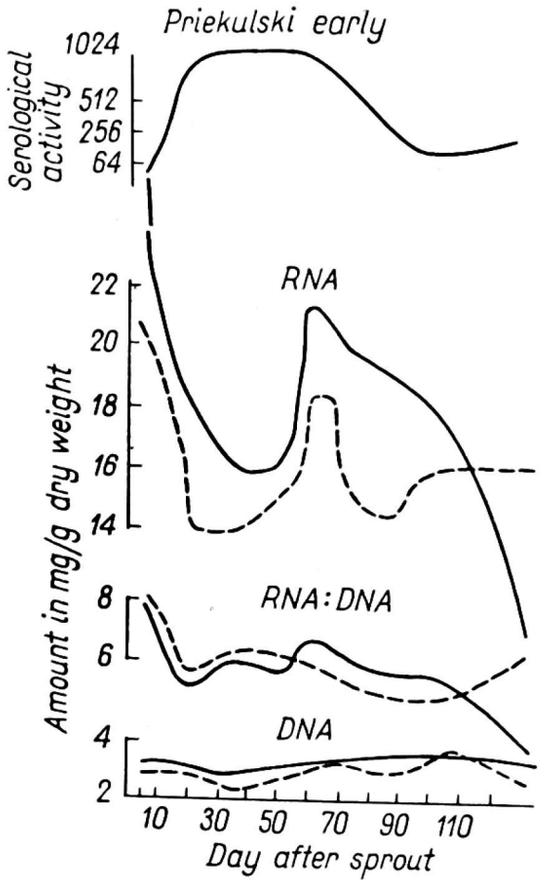

Fig. 3. Changes in nucleic acid content in leaves of potato variety Prijekulski early, grown from healthy tubers and from tubers, derived from plants, infected with the X_k strain during 3 consecutive years of cultivation, at different stages of infection. Explanations as in Fig. 1 and in text.

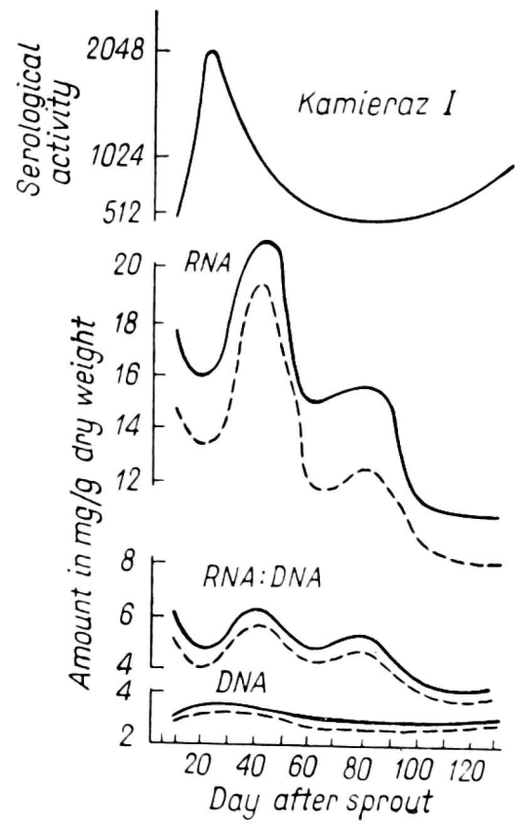

Fig. 4. Changes in nucleic acid content in leaves of potato variety Kamieraz (the semi late variety), grown from healthy tubers, and from tubers, derived from plants, infected with the X_k strain during three consecutive years of cultivation at different stages of infection. Explanations as in Fig. 1 and in text.

post infection. As was mentioned above, in case of infection with the X_s strain, no difference was found in the amounts of total RNA between healthy and virus diseased plants at the 5th day after inoculation. However there was a slight decrease in amounts of both AMP and UMP nucleotides, which apparently was counterbalanced by a simultaneous increase in CMP. At the same time, infection with the X_k strain did not cause any significant change in contents of all the four nucleotides.

At the 17 day after infection, when there is a culmination in both symptom expression and virus multiplication in the infected tissues, the increase in total RNA was most obviously correlated with the increase of CMP content. This increase was especially evident in plants infected with the severe virus X strain

C. PROTEIN CONTENT AND ITS AMINO ACID COMPOSITION

Determinations of the total and protein bound nitrogen, carried out simultaneously with nucleic acid analyses, have shown, that the amount of this element increases in virus infected plants at the 5th day after infection, regardless of the strain used for inoculation. In later stages however of the disease the level of nitrogen in virus infected plants depends both on the inoculated plant species and on the virulence of the applied virus X strain. As far as potato is concerned, this increase

in nitrogen was most pronounced in those plants which were infected with the severe X_s strain. In comparison with other plants used in the experiment the increase in nitrogen was highest in potato also after infection with other virus X strains [7].

Analyses of amino acid composition of proteins revealed certain reconstitution abilities regarding alanine synthesis in virus infected plants. At the 5th day after infection this amino acid accumulated in much greater amounts in the diseased plants of both *Datura* and the potato variety Prijekulski early, that in healthy plants of both species. However already after 10 days from the moment of inoculation, a tendency of decrease of the alanine content in virus infected plants, could be observed, this decrease being most conspicous after 20 days. If one considers, that the intense development of the disease usually begins 5 days after infection, and that it attains its culmination after 20 days, it is clear, that the observed abrupt change in alanine content at those moments reflects certain essential shift in cell metabolism, induced by viral infection. This shift was especially evident in plants infected with the severe virus X strain (X_s). In *Datura* a similar tendency exhibited

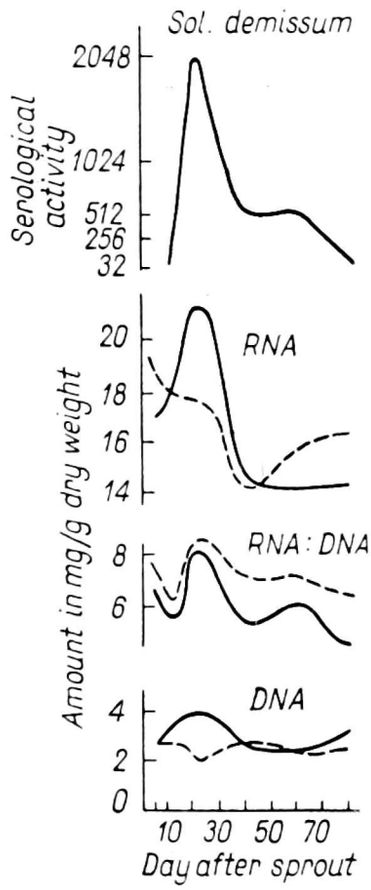

Fig. 5. Changes in nucleic acid contents in virus X (strain X_k) infected leaves of *Solanum demissum*, at different stages of infection. Explantion as in Fig. 1 and in text.

Table 1

Nucleotide composition of total RNA from *Datura* leaves infected with three different strains of the potato virus X (in mg per gram dry mass)

Nucleotides	5 day post infection				17 day post infection			
	healthy	X_r	X_k	X_s	healthy	X_r	X_k	X_s
CMP	13.1	10.5	12.87	19.92	6.44	9.02	21.6	19.17
AMP	19.07	10.17	19.91	15.55	10.58	6.89	9.21	9.1
UMP	8.00	8.9	9.77	3.83	3.08	2.53	3.74	2.21
GMP	10.31	11.11	14.02	12.02	8.32	10.86	13.1	10.3

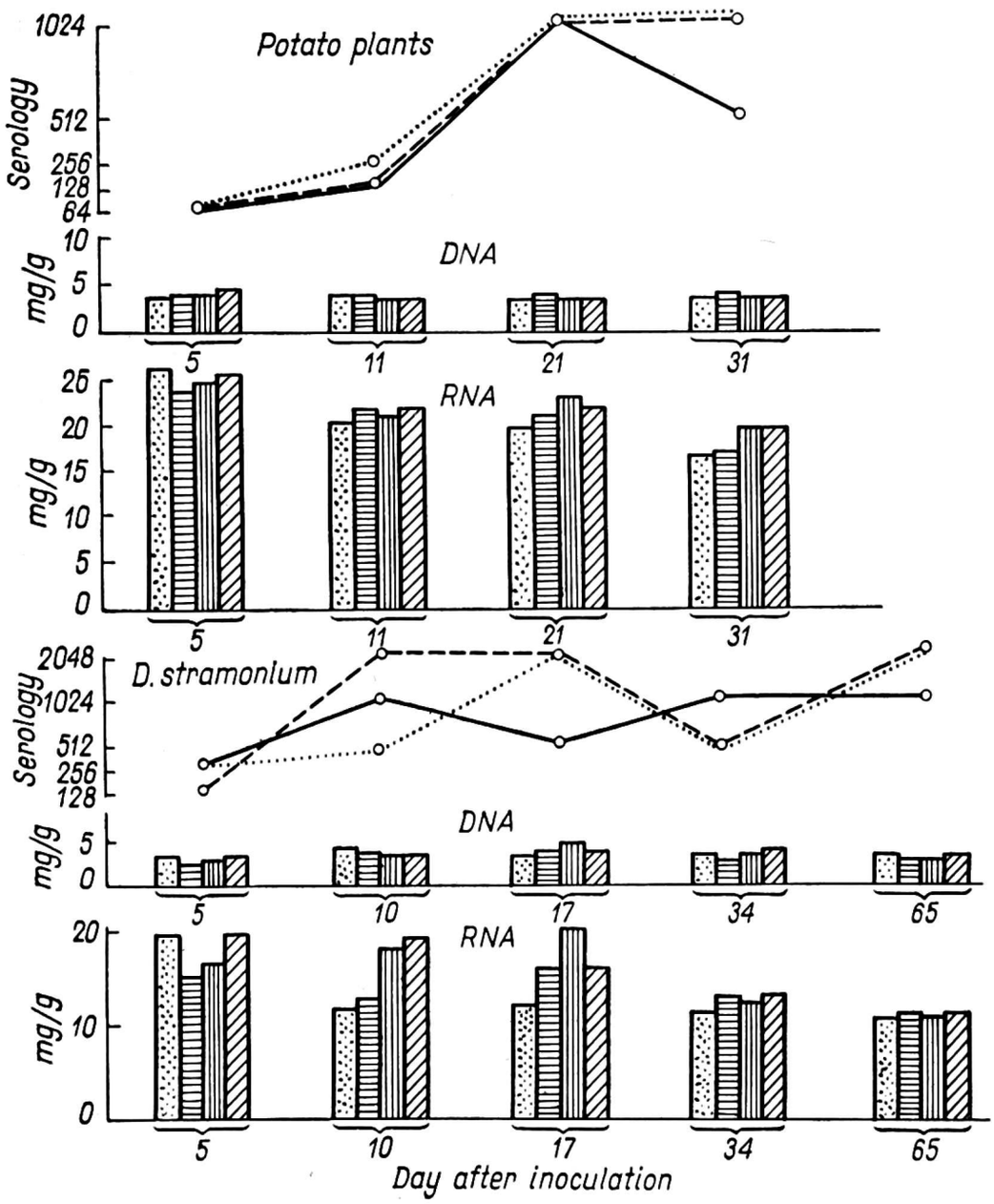

Fig. 6. Changes in nucleic acid contents in potato seedlings variety Prijekulski early, and in *Datura stramonium* at different stages of infection with the potato virus X (three strains). On each graph in the upper part the curves represent virus titer. Below RNA and DNA contents are represented by columns. In the titer curves solid line represent the X_r (mild) strain, the broken line — the X_k (Kijev) strain, and the dotted line — the severe, X_s strain. The black columns represent healthy (control) plants, columns with horizontal dashes — the X_r strain, columns with vertical dashes — the X_k strain, and with skew dashes — the X_s strain.

the aspartic acid, while an opposite tendency was stated in proline, tyrosine and glutamic acid contents.

It was also shown, that in the potato variety Prijekulski early there was a decrease in the contents of arginine and leucine + iso-leucine at the 20 day after inoculation what coincided with the maximum accumulation of virus material in the infected plant. This decrease was proportional to the degree of virulence of the applied strain. No regularity could be stated in changes of contents of other amino acids. The infected *Datura* plants exhibited in the moment of maximal viral synthesis (10 day after infection) an increase in lysine, threonine and proline contents proceeding parallely to the degree of strain virulence.

At the end of the vegetation period the virus infected plants revealed a distinct

increase in contents of aspartic acid, serine, proline, tyrosine, and leucine + iso--leucine, as well as a simultaneous decrease in histidine, phenylalanine and glutamic acid. The decrease in histidine and glutamic acid contents was most pronounced in plants infected with highly virulent virus X strain, and especially in *Datura*, which was tested in the last turn.

Purified virus X preparations contain in comparison with total plant protein much more aspartic acid, proline, alanine and phenyl-alanine [9]. It was shown in the above experiments, that the increase in contents of particular amino acids in virus protein proceeds always at the expense of a simultaneous decrease of those compounds in normal plant proteins. This correlation was true for all the amino acids discussed above. On the other hand a low amount of cystine in a purified virus X preparation coincides with an increase of this amino acid in normal proteins of plants infected with this virus.

THE CHANGES IN CELL ULTRASTRUCTURE CAUSED BY THE INFECTION WITH THE POTATO VIRUS X

Examination of ultra thin sections of the same tissue samples which were taken for biochemical analyses, in the electron microscope, revealed considerable changes in the ultrastructure of cells infected with the potato virus X, already at the 4-6th

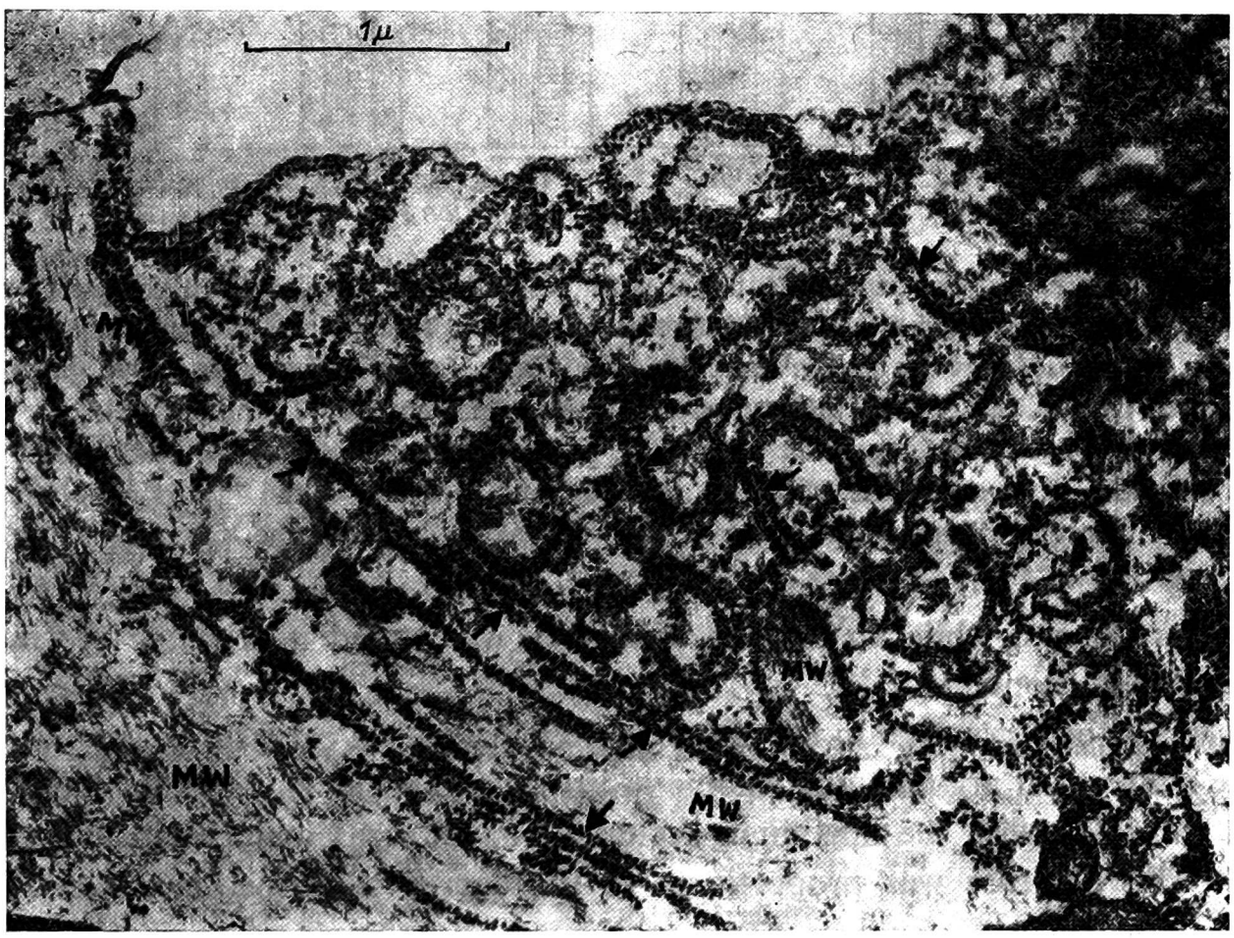

Fig. 7. Linear ribosomal groups in the X-body and virus material (MW) in parenchymatic cells of leaf of the potato variety Kamieraz I, infected with the X_s strain of the potato virus X. The arrows denote electron-dense threads, around which accumulate ribosomes. The scale represents magnification of 1 micron.

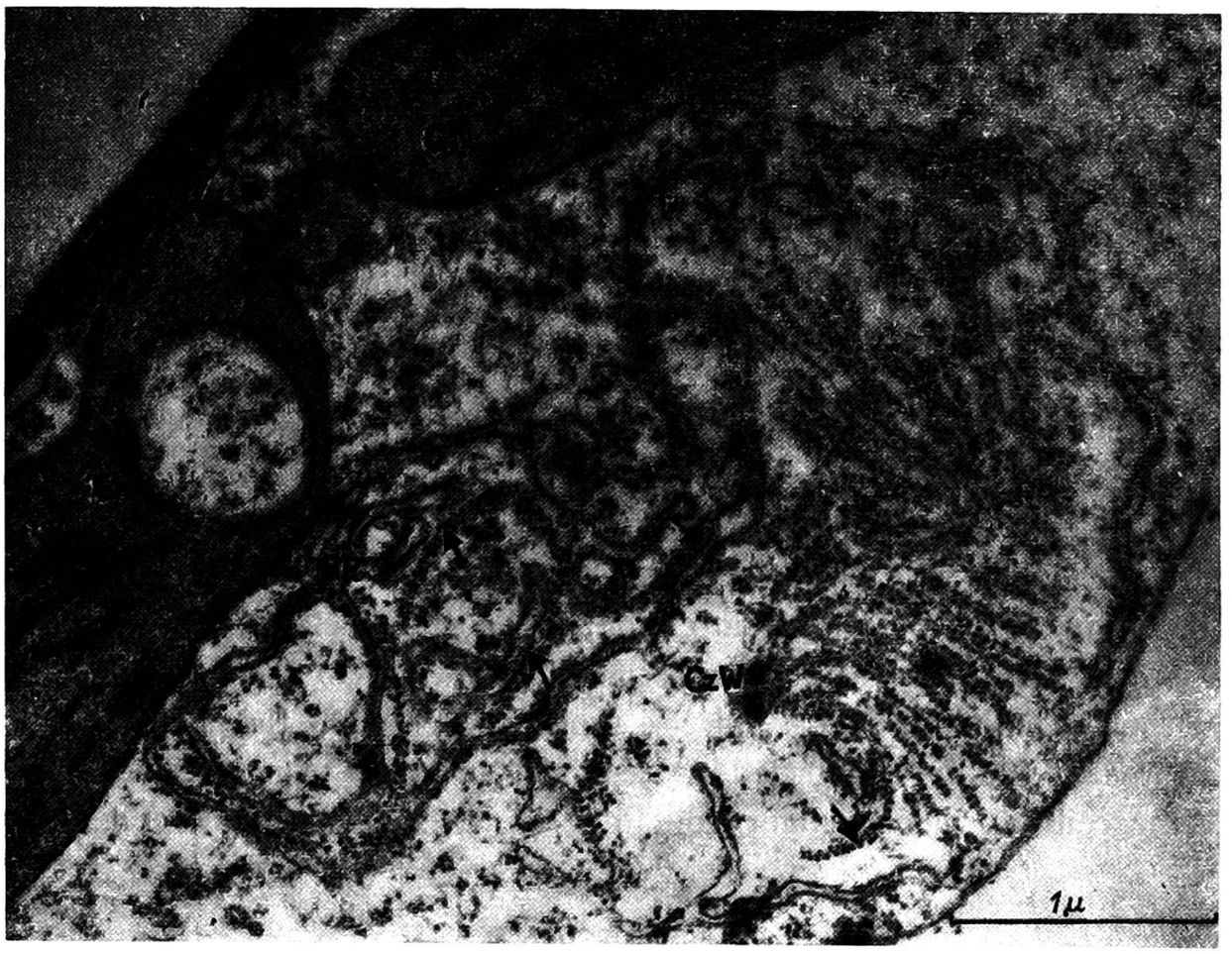

Fig. 8. Fragment of *Datura* leaf cell, infected with the X_k strain (taken from the chlorotic area). Fragments of endoplasmic reticulum (ER) follow the ribosomal groups with the electron-dense thread in the centre (marked with arrows), and complete visus particles (CzW). In one of the chloroplasts (Ch) a cytoplasmic invagination is visible enclosed in a double membrane. It is seen quite clearly, that that the electron-dense thread in the middle of linear ribosomal groups is much thinner and differs also in its appearence from ER membranes. The scale represents magnification of 1 micron.

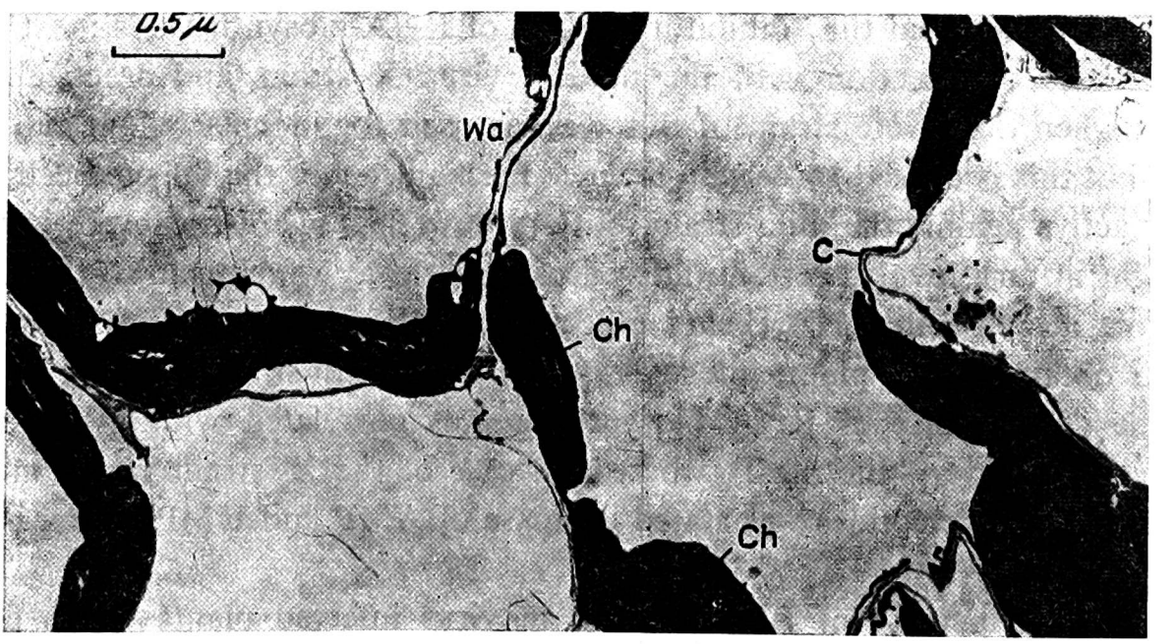

Fig. 9. Ultra-thin section through healthy, parenchymatic cell of *Datura* leaf. One can see, that the whole cell is occupied by a large, central vacuole (Wa), and only at the cell wall, there is a thin layer of cytoplasm, chloroplasts and other cell organelles. The scale represents magnification of 0.5 micron.

day after infection. Independently of the strain used for inoculation, intracellular inclusions, the so called X-bodies appear at this time in great quantities in the leaf cells, followed by increasing masses of virus material, which finally form the so called virus inclusions [10, 11]. In the case of the virulent strain X_s, those inclusions were localised in the vicinity or inside chloroplasts. Virus particles could be detected inside the plastids in form of fibrous masses with or without a double envelope. One can assume, that in this last case chloroplasts directly participate in virus reproduction [12].

New, important elements were detected in the ultrastructure of X-bodies. Using the glutar-aldehyde and osmic acid [13] it was possible to visualise inside those inclusions linearly arranged groups of ribosomes with viral particles in between. In the center of such groups there was always visible an electron-dense thread, both in *Datura* (Fig. 8) as in potato (Fig. 7). The thickness of those threads was 30-40 Å, what means, that they were much thinner, than the membranes of the endoplasmic reticulum (Fig. 8), but 3-4 times thicker, than the RNA strand. No such structures could be detected in sections prepared from the healthy plant tissue, the cells of which usually contain a large, central vacoule (Fig. 9).

DISCUSSION

The general tendency of variation in total RNA content of virus infected plants could not be regarded as accidental, because it appeared in every set of experiments, and every analysed sample.

The time of decrease in RNA content at the 5th day after infection coincides with the beginning of mass reproduction of infectious viral particles. It can be therefore regarded as a result of a fundamental shift in cell metabolism, which was induced by viral infection.

It is interesting, that the "inhibitory" effect of RNA biosynthesis is most pronounced in case of infection with the mildest virus X strain, and practically does not exist when the severe strain of this virus is used for inoculation. It can be assumed, that this is caused by certain defence mechanisms of the infected cell, which are only active against the mild strain, but are powerless against a severe infection. This might in turn involve certain differences in pathway which lead to the mentioned above decrease in RNA, being reflected in different nucleotide composition of RNAs obtained from plants infected with either of both strains. In case of the X_r strain the "inhibition" of RNA biosynthesis was correlated with a decrease of only one nucleotide — the AMP, whereas in case of the X_s strain variation in the content of three nucleotides was stated decrease of AMP and UMP, and a increase of CMP.

The relative increase of RNA content in virus infected plants which attaines its maximum 17-20 days after infection, coincides with the maximal accumulation of viral particles in the tissues and with the most distinct symptoms expression. While comparing those facts it becomes evident, that the increase in RNA content of virus infected tissues reflects the increase of viral RNA. If this is correct, we may

expect, that the nucleotide composition of RNA extracts at this time should be similar to that of the viral RNA, and to a much lesser extent to the cell RNA. Data obtained in the experiments did not however confirm this supposition. The RNA increase in extracts of virus infected plants is mainly due to the increase of CMP content, whereas PVX—RNA is characterized by a high AMP content [15]. It seems therefore more likely, that this observed increase of RNA content in virus diseased plants is caused by certain abnormal biochemical processes, which lead to the synthesis of CMP-containing RNAs.

The characteristic fall in RNA level of virus infected plants at the end of the vegetation period may be connected with the general recess of nutrients from leaves to the underground parts of the plant. This was confirmed by our observations, that this decrease in RNA was most prominent in plants with short vegetation periods, such as *Datura* and the early potato variety: Prijekulski early, but was almost invisible in semi-late potato variety Kamieraz I.

The DNA content seems to be independent of the infectious process, at least as far as potato virus X is concerned. This confirms the generally known idea, that DNA is one of the most stable compounds in the living cell.

Determinations of total protein content as well as its aminoacid composition did not reveal any clear influence of the infectious process. One can only say, that the observed variations in the contents of those compounds point out for a high lability of that important substance and require further, more detailed investigation. Certainly investigations of different groups of cell enzymes active during virus infection would open here most promising perspectives.

The relative decrease in cell protein of those amino acids which play the main role in the construction of virus protein might be interpreted as caused by the migration of viral particles out of the inoculated leaves. Virus protein is obviously synthesized on account of normal cell protein, and as such it migrates to other parts of the infected plant.

Certain authors [16, 17] suppose, that virus synthesis occurs mainly within the X-bodies. Results of our investigations, which revealed linear ribosomal conglomerates in those inclusions strongly support this view, indicating, that they might be at least indispensable for viral reproduction. Such conglomerates are well known from cells, which are in an active, metabolic state. The ribosomes are there connected along a mRNA strand and serve for synthesis of polypeptide chains [18]. The length of viral RNA particle is much greater, than lengths of other types of cell RNAs — it amounts to 10 μ [14]. It is therefore quite possible, that the reading out of information from such long RNA strands requires special ribosomal structures, which we have identified previously as "gigantic polysomes" [12, 14]. We have not found in the literature any other interpretation of the role of those structures in plant cells.

Apart of polysomes one can see within the X-bodies accumulation of virus particles and mitochondria. In the majority of cases the X-bodies are formed around the nucleus. At early stages of infection (in the 6th day) masses of virus particles could be detected mainly within the X-bodies, where they are often connected

with the described above polysomes. All those facts support our supposition, that the X-bodies play an active role in the synthesis of potato virus X, probably as sites of formation of polysomic structures. As was stated above, the electron dense strand in the center of those abnormal structures can not be identified on the ground of estimated parameters as endoplasmic membrane nor as single RNA strand. Actually the author has undertaken additional investigations of the biochemical and cytochemical character, with the aim to elucidate the nature of those structures.

CONCLUSIONS

It was shown, that in the course of infection, evoked by different strains of potato virus X, the decisive moment appeares on the 4-6th day after inoculation. At this time certain basic biochemical changes occur in the infected cell together with the appearence of abnormal ultrastructures and complete virus particles. The intensity of those changes directly depends on the severity of the applied virus strain.

It seems quite possible, that certain steps of virus X reproduction, such as biosynthesis of viral protein, require special configuration of cell ribosomes, which were previously identified as linear ribosomes groups or gigantic polysomes.

SUMMARY

The paper contains results of complex investigations, which were carried out by the author and his coworkers on protein and nucleic acid metabolism, as well as on changes in cell ultrastructure of different host plants, induced by the infection with three different strains of the potato virus X.

It was shown, that the content of RNA depends on the severity of the virus strain as well as on the stage of development of the infected plant and the course of virus multiplication in the infected tissues. At the 4-6th day after infection a distinct fall in the amount of RNA in virus infected plants as compared with the healthy ones, could be always observed. This fall was especially evident in plants infected with the mild PVX strain and it coincided with the beginning of mass reproduction of viral particles. It was also correlated with fluctuations in nucleotide composition induced by different strains. It is supposed, that these fluctuations reflect some defense reactions of the infected plant against virus invasion.

During the whole period of intense virus multiplication the RNA content in the infected plant exceeds much that in the control series. This increase in RNA proceeds parallely with a relative increase in CMP content.

At the end of the vegetation period there is a second decrease of RNA in the diseased plants, what is probably connected with the transport of RNA containing compounds, including viral particles, from leaves to the underground parts of the plant.

No essential changes in DNA content could be stated in virus infected plants in comparison with healthy ones.

Infection with the potato virus X induced however different fluctuations in protein and amino acid contents, which not always could be correlated with the investigated changes in genotype. The diseased plants usually contained more proteins, than the healthy ones, and their amino acid composition was much different in comparison with proteins of purified virus preparations. This could be interpreted as caused by the usage of cell protein resources for the synthesis of viral particles, as well as its migration from leaves to other parts of the infected plant.

At the 4-6th day after infection (the end of the vegetative phase of virus development), there appeared in the infected cells many virus inclusions in form of the so called X-bodies, followed by mass accumulation of viral material, whithin the X-bodies abnormal ultrastructures, composed of electron-dense threads with ribosomal conglomerates — which were called "gigantic polysomes" — were detected and described for the first time. The author assumes, that these facts prove the active role of the X-bodies, as well as the metioned polysomic structures in potato virus X reproduction, or at least in biosynthesis of its components.

Finally, it was stated, that the decisive moment in the development of the infectious process induced by potato virus X, takes place 4-6 days after infection. At this time the intensity of virus induced changes in the metabolism of infected plant, directly depends on the virulence of the applied strain.

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