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Cadmium Ions as Inhibitors of Tobacco Mosaic Virus

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Souhrn

Účinek kademnatých iontů na reprodukci viru tabákové mosaiky byl studován na rostlinách tabáku (*N. tabacum* Samsun), které byly pěstovány ve skleníku v kořenáčích. Pokusné rostliny byly zalévány roztokem síranu kademnatého o koncentraci 4 mg Cd^{2+}/1 ml v různých dávkách. U každé rostliny byl očkován jeden list Al-kmenem VTM. Optimální dávka kademnatých iontů, která nemá žádný škodlivý vliv na pokusné rostliny, je dávka 160 mg Cd^{2+} na jednu rostlinu aplikovaná ve dvou zálivkách, a to těsně před inokulací a třetí den po inokulaci. Reprodukce VTM je inhibována ze 70 % podle testu na *N. glutinosa* a hypersensitivním hybridu. Inhibiční účinnost kademnatých iontů značně poklesne při zálivce roztokem kademnatých iontů 24 hodiny před inokulací. Z rozdílů v průběhu primární infekce, sledované jodovou zkouškou, jsou nápadné zvláště tyto skutečnosti: u pokusných rostlin se ztrácí škrob z infikovaných listů pomaleji, vývoj chlorotických skvrn je zbrzděn a jejich ohraničení a struktura nejsou ostré. Vyšší dávky kadmia (kolem 300 mg na jednu rostlinu) vyvolávají na některých listech mladých rostlin tabáku (*N. tabacum* Samsun) skvrny, které připomínají nekrotické lese na listech *N. glutinosa* po infekci VTM. Předběžně bylo zjištěno, že kademnaté ionty vyvolávají poruchy v metabolismu fosforu. V diskusi je vyslovena domněnka, že inhibiční účinek kademnatých iontů na reprodukci VTM může spočívat, analogicky k představě vytvořené v živočišné fysiologii, v interakci iontů zinku a kadmia v metabolismu nukleoproteinů.

Summary

1. While studying the inhibitory effects of cadmium ions on the reproduction of tobacco mosaic virus in vivo it was found that a dose of 160 mg. Cd^{2+} applied in two waterings to one plant (immediately before inoculation and

the third day following inoculation) produced an average 70% inhibition, without in any way injuring the plants themselves.

2. The inhibitory effect of cadmium ions decreased considerably when the plants were watered with a cadmium ion solution 24 hours before inoculation.

3. The differences in the course of primary infection, as followed by means of iodine tests, were particularly apparent in the following respects: starch is lost from the infected leaves of the experimental plants more slowly, the development of chlorotic spots is slowed down and their delimitation and structure are not so sharp.

4. On some leaves of young tobacco plants (*N. tabacum* Samsun) larger doses of cadmium produce spots, which resemble the necrotic lesions on leaves of *N. glutinosa* following infection with TMV.

5. Preliminary determination indicates that cadmium ions cause a disturbance of phosphorus metabolism.

6. In the discussion it is suggested that the inhibitory effect of cadmium ions on the reproduction of TMV may, analogically to the conception existing in animal physiology, be connected with the interaction of zinc and cadmium ions in nucleoprotein metabolism.

Introduction

The effects of many different substances on the reproduction of plant viruses have been the subject of much work, which has been summarized for instance by MATHEWS and SMITH (1955). Recently the greatest attention has been devoted to derivatives of purines and pyrimidines, which are also the only substances for which the mechanism of the inhibitory effect has been explained (MATHEWS and SMITH 1955). These derivatives block the biosynthesis of virus nucleic acids by becoming incorporated into the molecules in place of the natural purines and pyrimidines.

Of the series of metal ions which have been tested as agents against plant viruses (e. g. MATHEWS and SMITH 1955, RYZHKOV and MARCHENKO 1957), the most significant inhibition was found with zinc (STODDARD 1947, WEINTRAUB et. al. 1952, RUMEY and THOMAS 1951). The results are not, however, quite consistent. YARWOOD (1953, 1954) came to the opposite conclusion and demonstrated the stimulatory effect of zinc on the reproduction of tobacco mosaic virus. This contradiction was explained by HELMS and POUND's work (1955); they found that zinc affects the reproduction of tobacco mosaic virus in the same measure as the growth of the host. According to these authors, increased doses of zinc increase virus accumulation insofar as they stimulate the growth of the host.

The aim of the present work has been to find an effective chemotherapeutic agent, the application of which would, at a later stage of this work, make it possible to examine the mechanism of virus nucleoprotein biosynthesis. From the start the use of purine and pyrimidine derivatives was excluded, since these are substances the mode of action of which is known. On the basis of

Fig. 1a

Fig. 1b

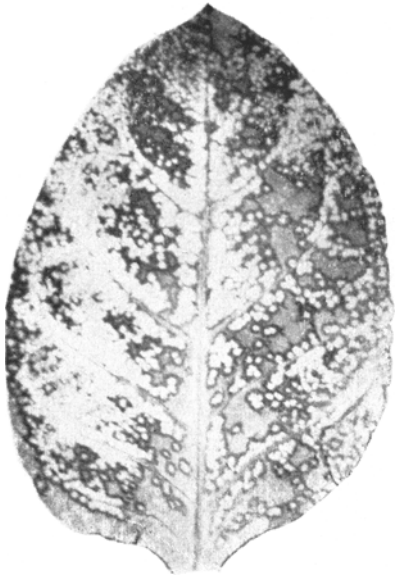

Fig. 2a

Fig. 2b

Course of primary infection, followed by the iodine test, on leaves of *N. tabacum* Samsun after inoculation with Al-strain TMV and watering with cadmium sulphate solution (dose 160 mg. Cd^{2+} per plant) divided between two waterings: immediately before inoculation and third day after inoculation.

Fig. 1. 3rd day after inoculation. a = control, b = experiment.

Fig. 2. 4th day after inoculation. a = control, b = experiment.

Fig. 3a

Fig. 3b

Fig. 4

Fig. 3. 5th day after inoculation. a = control, b = experiment.
Fig. 4. Necrotic spots on leaves of young *N. tabacum* Samsun plants after watering with cadmium sulphate solution (dose 320 mg. Cd^{2+} per plant). The spots resemble lesions on leaves of *N. glutinosa* after infection by TMV.

considerations which will be mentioned in the discussion, the experimental determination of the effect of cadmium ions on the reproduction of tobacco mosaic virus was undertaken. So far as is known to the author, the effect of this ion on the reproduction of plant viruses has not yet been studied.

Material and Methods

All experiments were carried out with tobacco plants (*Nicotiana tabacum* Samsun) at various stages of growth, cultivated in pots in the glasshouse.

Cadmium ions were applied in the form of sulphate. The experimental plants were watered with a solution of concentration 4 mg. Cd^{2+} in 1 ml. Doses of cadmium were tested within the range of 20 to 400 mg. Cd^{2+} to one plant. Watering with the experimental solution was carried out immediately and 24 hours before inoculation. In some of the experimental series plants were watered with the cadmium sulphate solution for a second time, on the third day following inoculation.

The Al-strain of tobacco mosaic virus (BLATTNÝ 1955) was used for inoculation because of its short incubation period. One leaf of each plant was inoculated, by the usual technique with carborundum. There were twenty plants in each variant of the experiment. The experiments were carried out from May to October 1958.

Following inoculation the course of primary infection was followed by means of the iodine test on starch (HOLMES 1931), in each variant on five leaves. The inoculated leaves were cut off, killed by plunging into boiling water, chlorophyll was extracted from them by boiling in 96% alcohol and they were then submerged in a dilute solution of iodine in potassium iodide.

On about the seventh day after inoculation, when the youngest leaves of the control plants began to show signs of systemic disease, the tips of the plants were cut off, homogenized and the relative virus concentration was determined by testing on *Nicotiana glutinosa* and on a hypersensitive hybrid[1]), which has been evolved by Květa Schwammenhöferová.

The content of free and labile phosphorus in tobacco leaves following watering with cadmium sulphate solution was determined orientationally. Phosphorus was determined by the modified method according to PONS and GUTHRIE (1946) and ZELLER (1949). Plant homogenates were deproteinized with 0·25 M trichloracetic acid.

Results

Figures 1 to 3 show the differences in the course of primary infection between experimental and control plants from the June series. It is clear from the figures that cadmium ions delay the reproduction of tobacco mosaic virus. The following facts are particularly striking: in comparison with the control the leaves of experimental plants lose starch more slowly, the development of chlorotic spots is slower and their delimitation and structure are not sharp.

The results of quantitative tests on *N. glutinosa* and the hybrid with necrotic reaction are summarized in table 1. This table shows that the optimal dose of cadmium ions, which has no injurious effect on the plants themselves, is a dose of 160 mg. Cd^{2+} to one plant, applied in two waterings, one im-

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[1]) Hybrid: Trapezond 161 × 8/37 (8/37—new selection VÚTP— Báb pri Seredi). This material was used for the quantitative testing of TMV for the first time. It was subjected to preliminary testing and results compared with those from *N. glutinosa*.

Table 1.

Date of experiment	No. of developed leaves on exp. plants	Dose of Cd^{2+} in mg. per plant	Watering with Cd^{2+} solution before inoculation	Inhibition %[1]	No. of lesions on half leaf of *N. glutinosa* or hypersensitive hybrid Average and mean error		P	n
					Control	Experiment		
21. 5.	8	60	immediately	45·0	183·1 ± 3 . 25·7	102·4 ± 3 . 10·6	10^{-2}	8
27. 5.	8	80 + 80[2]	immediately	71·5	63·8 ± 3 . 11·4	18·2 ± 3 . 4·1	10^{-4}	18
11. 6.	8	80 + 80	immediately	69·8	49·2 ± 3 . 9·2	14·7 ± 3 . 2·4	10^{-4}	18
13. 6.	8	130	immediately	61·2	32·5 ± 3 . 5·2	12·6 ± 3 . 2·2	10^{-4}	24
13. 6.	12	130	immediately	55·3	27·7 ± 3 . 5·8	12·3 ± 3 . 2·5	10^{-2}	19
15. 8.	9	80 + 80	immediately	64·0	107·6 ± 3 . 14·7	38·7 ± 3 . 5·4	10^{-5}	25
15. 8.	9	80 + 80	24 hours	5·7	83·0 ± 3 . 8·9	74·2 ± 3 . 7·8	$3 . 10^{-1}$ [4]	25
15. 8.	10	80 + 80	immediately	67·8	531·2 ± 3 . 90·6	170·8 ± 3 . 28·6	10^{-4}	15
15. 8.	10	80 + 80	24 hours	46·9	353·8 ± 3 . 91·4	187·6 ± 3 . 50·5	10^{-1} [4]	15
28. 9.	8	80 + 80	24 hours	19·4	270·2 ± 3 . 24·7	217·7 ± 3 . 19·6	10^{-1} [4]	19
29. 9.	8	400[3]	immediately	83·7	85·6 ± 3 . 23·8	13·9 ± 3 . 3·2	10^{-3}	11
1. 10.	4	160 + 160	immediately	76·3	50·3 ± 3 . 6·1	11·9 ± 3 . 1·4	10^{-9}	25

[1]) Calculated according to UTECH and JOHNSON (1950).
[2]) Second watering carried out in all series on the third day.
[3]) Concentrations inhibiting plant growth.
[4]) Not statistically significant. Statistical methods according to HRUBÝ (1950) were applied.

mediately before inoculation and the other on the third day after inoculation. Inhibition of tobacco mosaic averaged 70%. It is interesting that the inhibitory effect of cadmium ions was found to decrease considerably when the experimental plants were watered with cadmium sulphate solution 24 hours before inoculation.

Larger doses of cadmium ions, about 250 mg. to one plant, inhibit growth slightly. Naturally, a very important factor is the age of the plant. Following watering with cadmium ion solution containing a 320 mg. dose, necrotic spots (fig. 4) appear on the leaves of young tobacco plants (*N. tabacum* Samsun). These resemble the necrotic lesions on the leaves of *N. glutinosa* following inoculation with TMV.

The results also show that tobacco plants can stand a relatively high dose of cadmium without injury if the solution is applied by watering. The question of the amount of cadmium ions that get into the plant remains open.

When the leaves are sprayed necrosis appears on them even with concentrations of 1·5 μg. Cd^{2+} in 1 ml. This fact also did not permit direct proof to be given that inhibition of the virus in the inoculum is not involved. The results obtained while following the course of primary infection are regarded as sufficient in this respect (fig. 1 to 3). The number of chlorotic spots on the inoculated leaves of the experimental plants was the same as in the control.

In preliminary experiments changes in the content of inorganic and labile phosporus in tobacco leaves following watering with cadmium sulphate solution were followed. Although the methodic aspect of these experiments is very difficult, because the age of the plants must be taken into account and watering does not allow of exact dosing with cadmium, these orientational experiments seem to indicate that the smaller doses of cadmium ions increase the content of inorganic phosphorus; on the contrary, larger doses, which delay plant growth, decrease the free phophorus content to nearly one half. It will be possible to arrive at a final conclusion on this question only after a thorough analysis has been made.

Discussion

A number of investigations have been carried out on the effect of cadmium on higher plants (e. g. SCHARRER and SCHROPP 1934, SPENCER 1937, SCHARRER 1941). Most of these have been carried out from the point of view of plant nutrition, but none of them deals with the physiological effects of this element. All authors agree that cadmium ions are definitely more poisonous than zinc ions.

PAŘÍZEK (1956, 1957) found that cadmium is a very strong poison for male gonads, i. e. organs with a very intensive nucleoprotein biosynthesis and with relatively high zinc content. In the view of the above author, cadmium injures the testes because it excludes zinc, which is linked to nucleoprotein metabolism as a biogenic element. Following the application of cadmium, intensive decomposition of nucleotides takes place and phophorus is freed and washed out of the bodies of the experimental animals.

Very little is know as yet about the biological function of zinc ions in plants (SCHARRER 1941, CHESTERS and ROLINSON 1951). The most typical symptoms of zinc deficiency are disturbances in chlorophyll synthesis and dwarf growth. Recently FUJII (1956) found that some cells, e. g. the staminal hair cells of *Tradescantia*, contain a relatively high content of zinc at the prophase. The last-mentioned facts would seem to indicate that zinc in plants is intimately connected with growth and cell division and that it is most probably involved in nucleoprotein metabolism, and therefore also in virus biosynthesis. This assumption is finally supported by the work of HELMS and POUND (1955), as mentioned in the introduction. They show the dependence of growth of the host and virus reproduction on dosing with zinc.

The results obtained so far from experiments on the inhibitory effect of cadmium ions on TMV reproduction do not permit the author to draw any conclusions regarding the mechanism of the inhibitory effect of these ions. One of the possible hypotheses, which might be adopted, is an analogy with the conception put forward in animal physiology that cadmium displaces zinc, which is linked with nucleoprotein metabolism. The alternative cannot, however, be excluded, i. e. that cadmium as a chemically related element can to a certain extent compensate for or take the place of zinc.

In conclusion it may be said that further study under more exact conditions of the already established inhibitory effect of cadmium ions on the reproduction of tobacco mosaic virus could contribute not only to a deeper understanding of the mechanism of the biosynthesis of virus nucleoproteins, but also to the explanation of the physiological effect of cadmium ions and of the biological function of zinc ions.

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Ионы кадмия — ингибитор вируса табачной мозаики

МАРИЕ УЛРЫХОВА-ЗЕЛИНКОВА

Резюме

Изучалось действие ионов кадмия на репродукцию вируса табачной мозаики на табаке (*Nicotiana tabacum* Samsun), выращиваемом в горшках в оранжерее. Подопытные растения поливались раствором сульфата кадмия концентрации 4 мг Cd^{2+}/1 мл в разных дозах. У каждого растения инокулирован один лист Al штаммом ВТМ. Оптимальной дозой ионов кадмия, не имеющей отрицательное влияние на подопытные растения, является доза 160 мг Cd^{2+} на одно растение, примененная в двух поливах, как раз перед инокуляцией и на третий день после инокуляции. Репродукция ВТМ ингибирована из 70% по результатам теста на *N. glutinosa* и гиперсенситивном гибриде. Ингибирующее действие ионов кадмия значительно понижается в случае поливки раствором кадмия 24 часа до инокуляции. Из различий хода первичной инфекции, изучаемых при помощи йодного теста, особенно знаменательны следующие факты: подопытные растения теряют из зараженных листьев крахмал медленнее, развитие хлоротических пятен заторможено и структуры не резкие. Завышенные дозы кадмия (около 300 мг на одно растение) вызывают на некоторых молодых растениях табака (*N. tabacum* Samsun) пятна, напоминающие некротические пятна на листьях *N. glutinosa* после заражения ВТМ. В качестве предварительных данных установлено, что ионы кадмия вызывают нарушения в метаболизме фосфора. В дискуссии сформулирована гипотеза, что ингибирующее действие ионов кадмия на репродукцию ВТМ может базироваться — по аналогии с представлениями в физиологии животных — на взаимодействии ионов кадмия и цинка в метаболизме нуклеопротеинов.