Substances disturbing the *in vitro* coagulase activity

Substance	Minimal concentration causing coagulase inhibition in γ/ml
Sodium ethylmercurithiosalicylate	0·46
Albucid	0·46
Conserving solution	0·46 ml
Nucleic acid	0·46
Desoxyribonucleic acid	0·46
$CuSO_4$	1·85–7·82[a]
Na_3N	7·82–31·25[a]
$K_2Cr_2O_7$	31·25–125·0[a]
$ZnSO_4$	125·0
$CdSO_4$	215·0– 250·0[a]
$MgCl_2$	125·0–1000·0[a]
Phenol	125·0–1000·0[a]
$H_3P/Mo_3O_{10}/_4$	125·0–1000·0[a]
$PtCl_3$	500·0
$BaCl_2$	500·0
$HgCl_2$	500·0
KJ	500·0
$Al_2/SO_4/_3$	500·0
Sulfamethoxypirydazine	500·0
NH_4Cl	500·0–1000·0[a]
$K_4/Fe/CN/_6$	1000·0
Sulfanilamide	1000·0
Oleandomycin	1000·0
Spiramycin	1000·0

[a] In some cases the degrees of inhibition of coagulase in supernatant and living culture–differed.

sulfanilamide, which acted in the highest concentration and sulfamethoxypiradazine, which inhibited clotting also in a rather great quantity (500 γ/ml). The most marked action among antiseptics was that of sodium ethylmercurithiosalicylate ('thiomersalate', 'merthiolate'). This compound even in the lowest quantity (0·46 γ/ml) completely prevented clotting. The action of phenol and rivanol was slightly inhibitory.

The preserving solution used at the blood banks for transfusion purposes (it is mixed in proportions 1:5 with blood and consists of 30 g of sodium citrate, 30 g of glucose, 5 g albucid, 0·03 g rivanol, 22 ml of 50% HCl, and distilled water to 1000 ml, pH = 4·9–5·1)[4] was strongly inhibitory to coagulase. Even 0·46 ml of it prevented the clotting. It is rather interesting because all tests connected with physiological blood clotting were correct.

Nucleic acids also completely inhibited the coagulase action. Dextrans, with the exception of two samples which slightly delayed the clotting process, did not inhibit the coagulase.

Out of 29 inorganic salts, only 17 influenced coagulase at rather higher concentrations [NH_4Cl, $ZnSO_4$, $CdSO_4$, $Al_2(SO_4)_3$, $CuSo_4$, $K_4Fe(CN)_6$, KJ, $HgCl_2$, Na_3N, $BaCl_2$, $MgCl_2$, $K_2Cr_2O_7$, $FeCl_3$, $PtCl_3$, and $H_3P(Mo_3O_{10})_4$]. Some differences between the inhibition of coagulase concerned in the supernatant and living culture were observed in the experiments with salts.

The mechanism of these inhibitions is unknown in relation to the action of coagulase and physiological blood clotting.

Detailed results and a critical review on the problem described will be published later[5].

J. Jeljaszewicz and K. Wlodarczak

Department of Microbiology, Medical Academy, Poznań and Regional Sanitary and Epidemiological Station, Poznań (Poland), February 4, 1960.

Zusammenfassung

Es wurde der Einfluss von 67 Substanzen (Antibiotika, Chemotherapeutika, Sulfonamide, Antiseptika, Dextrane, Nukleinsäuren und Metallsalze) auf die Aktivität der freien Koagulase der Staphylokokken untersucht, worunter 24 als Inhibitoren auftraten.

Glutamic Acid-Alanine Transaminase in Tobacco Leaves Infected with TMV

In one of the former works, active glutamic acid-alanine transaminase and decarboxylase of glutamic acid in leaves of tobacco and other plants was proved[1].

Later on it was found that decarboxylase, in plants inoculated with TMV, increases in intensity[2].

Here the preliminary results are shown dealing with investigation in transamination in tobacco inoculated with tobacco mosaic virus (TMV). The method of investigation was similar to that described in the former account, and therefore only the most important finding are given here.

A sample (2 g) of leaves, picked in the morning, was ground in a mortar together with 5 ml *M*/15 phosphate buffer pH 7·6. The homogenate thus prepared in a quantity of 3 ml was mixed in a test tube with 2 ml glutamic acid (concentration 0·02 *M*) and 2 ml of pyruvic acid of the same concentration. The sample was divided into two parts; one of which was immediately boiled and the other with a delay of 45 min after incubation at 27°C. After boiling, they were filtered and the product was pipetted onto Whatman No. 1 filter paper and chromatographed in 80% phenol.

The activity of transamination was estimated by the increase of alanine. The chromatograms were coloured with ninhydrin (0·5) and the coloured areas which developed were eluted; measurements were made using Pulfrich photometer according to Kretovich[3].

The tested plants was *Nicotiana tabacum* v. White Burley, grown in a glass-house. It was inoculated with TMV and with the use of carborundum, according to the generally accepted rule.

The results are shown in the diagram. It is evident that, on the second day after inoculation, the transamination increases greatly. On the third day, it drops more than 50% in comparison with sound leaves. The activity of glutamic-alanine transaminase remains on that level until the mosaic appears on the leaves. Later on (4 weeks after inoculation) it increases to 75%.

Nowadays, the generally accepted conviction is that transamination is the cardinal process in the synthesis of amino acids and the inhibition of it may be incomprehensible in the case of plants synthetising TMV.

After the inoculation of tobacco with TMV, its leaves become yellowish day by day. It may be the evidence of decreased activity of some metabolic processes. On the other hand, however, intensive virus synthesis and increased activity of some enzymes[4] and processes are to be observed[5].

It is very likely that inhibition of transamination is due to a peculiar change of plant metabolism caused by the synthesis of extraneous proteins.

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Investigations to elucidate the process and its connection with virus synthesis are going on.

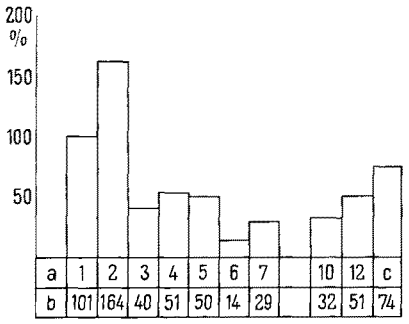

Fig. 1. Activity of transaminase on dry weight

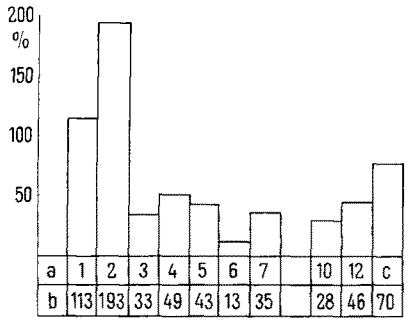

Fig. 2. Activity of transaminase on fresh weight
a) Days after infection b) Percentage of transamination in inoculated leaves in terms of the activity of sound plants (100%). c) 4 weeks after infection.

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M. GUBAŃSKI and R. KURSTAK

Department of Plant Physiology, University of Lódź (Poland), January 25, 1960.

Zusammenfassung

In den mit Mosaikvirus infizierten Tabakblättern wurden im Vergleich mit den gesunden bedeutende Veränderungen in der Glutamin-alanin-transaminase-Aktivität festgestellt. Zwei Tage nach Infektion erfolgt zunächst Zunahme, dann Abnahme der Enzymaktivität.

10. On the Sulphurylation of Mono-, Di-, and Trihydric Phenols

The sulphurylation of a large number of phenols and phenol derivatives has been studied by earlier workers. This has been done in *in vivo* experiments on various species[1], or *in vitro* in slices of various tissues[2,3], as well in particle-free media containing sulphate-activating and sulphate-transferring enzymes[4–7].

The last-mentioned technique was used in the present comparative study on the sulphurylation of a number of mono-, di-, and trihydric phenols. S^{35}-labelled sulphate was used as tracer. The formation of conjugated sulphates in the incubating medium was followed by means of two-dimensional paper chromatography and electrophoresis,

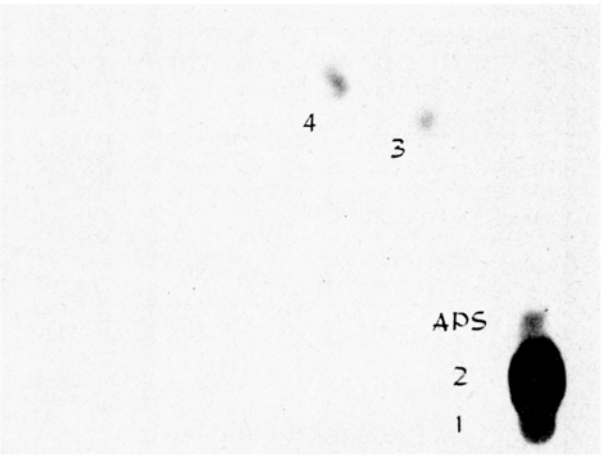

Fig. 1. Basic chromatographic pattern of the incubating medium. Spots 1 and 2 correspond to inorganic sulphate and active sulphate (PAPS) and spots 3 and 4 to unknowns derived from the liver extract.

both techniques combined with autoradiography on Gevaert X-ray film. Detailed descriptions of the methods used have been given elsewhere[8].

The following types of experiment were run:

(a) Incubation for 2 h at 37°C in the complete system (particle-free rat liver supernatant buffer[9], ATP, Mg ions and S^{35}-labelled sulphate) of various concentrations (0·1 to 2·0 *mM*) of each of the following phenols: phenol, catechol, resorcinol, hydroquinone, pyrogallol, and phloroglucin.

(b) Incubation as above of the complete system, with the phenol solutions replaced by water (control).

(c) Incubation as above of the complete system, with the S^{35}-sulphate solution replaced by water, and the phenol solutions replaced by S^{35}-labelled monosulphates of the aforementioned phenols obtained by biochemical synthesis.

(d) Acid hydrolysis in 0·1 *N* HCl for 0–2 h of eluted S^{35}-labelled spots obtained in the experiments listed above. The hydrolysates were submitted to paper chromatography and paper electrophoresis combined with autoradiography.

The results of these experiments are illustrated in Figures 1–3. The basic chromatographic pattern of the incubating medium in the control experiments (b), shown in Figure 1, was essentially the same as described earlier[8]. In addition to the inorganic + PAPS spot and the APS spot in the right lower corner, a few additional spots due to the sulphurylation of sulphate acceptors present in the rat liver supernatant are visualized. In the presence of

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