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ACTIVITY AND STABILITY OF ENZYME MOLECULES FOLLOWING THEIR CONTACT WITH CLAY MINERAL SURFACES

ŞTEFAN KISS*, MIHAIL DRĂGAN-BULARDA* and DANIELA PAŞCA**

SUMMARY. — The paper is a review of the literature data concerning inhibitory and stabilizing effects of clay minerals on enzymes. It consists of two parts. The first part, devoted to the inhibitory effect of clay minerals on enzymes, comprises 4 sections: activity of enzymes in the presence of clay minerals; activity of enzymes added to soil samples; activity of soil enzymes in clay mineral-soil mixtures; and activity of enzymes added to clay mineral-soil mixtures. The second part deals with the stabilizing and protecting effect of clay minerals on enzymes.

Literature data are grouped according to the nature of the studied enzymes and to the primary significance of the reaction catalyzed by them for one of the biological cycles of elements (C, N, P or S).

Finally, the need for further research to develop clay enzymology and the association of clay minerals with humic substances in enzyme accumulation in soil are discussed, and a model concerning distribution of accumulated and free enzymes in the soil microenvironment is presented.

Introduction

Maintenance and perpetuation of life on our Planet are conditioned by the biological cycles of elements (C, N, P, S, etc.), in which photosynthesis and decomposition of organic (plant, animal, microbial and xenobiotic) residues are the basic processes. In the decomposition of organic residues, the enzymes accumulated in soil — besides the enzymes of the proliferating microorganisms — play a key role. The clay minerals contribute to this accumulation [23, 24, 63, 118].

The paper reviews the results of researches proving that the contact between enzyme molecules and clay mineral surfaces leads, in general, to inhibition of enzyme activity, followed by stabilization and protection of residual activity*.

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* The results of researches on related topics such as *a*) adsorption or immobilization of enzyme preparations on clay minerals without assessing its effect on enzyme activity, *b*) catalytic power of clay minerals, *c*) enzyme activity of soil fractions of different particle sizes, *d*) adsorption of enzyme preparations on soil samples without assessing its effect on enzyme activity, *e*) microbial decomposition of enzyme preparations added to soil samples and incubated for many days, *f*) effect of proteolytic enzyme preparations on the natural enzyme content of soils and soil fractions, *g*) effect of proteo- and saccharolytic enzyme preparations on the enzymes isolated from soils, and *h*) use of enzyme preparations for analysis of some soil constituents will not be reviewed in the present paper.

Many clay minerals were studied. Some of them belong to the 1 : 1 layer silicates comprising the non-expanding kaolinite and halloysite and the amorphous allophane, and others to the 2 : 1 layer silicates comprising non-expanding (*e.g.* illite), expanding (*e.g.* montmorillonite) and fibrous (*e.g.* attapulgite) clays. The most frequently studied clay minerals were kaolinite, montmorillonite and bentonite (bentonite contains prodominantly montmorillonite).

The number of enzymes studied is also great. They belong to two classes: hydrolases and oxidoreductases.

I. Inhibitory Effect of Clay Minerals on Enzymes

The researches dealing with the inhibitory effect of clay minerals on the activity of enzymes may be grouped into 4 types, according to the source of clay minerals and that of the enzymes:

type 1: pure clay minerals are used together with partially or completely purified enzyme preparations originating from microorganisms, plants or animals;

type 2: enzymatically active or inactivated soil samples are used together with enzyme preparations;

type 3: clay mineral-soil mixtures are used, with the natural enzyme content of soil as the only enzyme source; and

type 4: clay mineral-soil mixtures are treated with enzyme preparations.

I.1. Activity of Enzymes in the Presence of Clay Minerals

I.1.1. *Enzymes of the carbon cycle*

I.1.1.1. *Invertase.* Adsorption of partially purified yeast invertase preparations to kaolin resulted in great activity losses. Consequently, it is not possible to use adsorption to kaolin for obtaining very pure invertase preparations (Willstätter and Schneider [125]). Bentonite was found to adsorb, at pH 2.9, the polymannan component (the stabilizer) of yeast invertase and to bring about a more or less rapid inactivation of the enzyme (Fischer *et al.* [40]).

There was a direct relationship between the amount of kaolinite and bentonite and their inhibitory effect on the invertase activity of yeast autolysate (Kiss [61]). Montmorillonitic clays (gumbrine, steatite, bentonite and nontronite) were stronger inhibitors of yeast invertase activity than kaolinite and monothermite (Zvyagintsev and Velikanov [128, 129]; Velikanov and Zvyagintsev [123]). Ross [109] also found significant decreases in yeast invertase activity in unbuffered and buffered clay suspensions. The inhibitory effect of clay minerals was in the order: montmorillonite > illite > kaolinite > allophane > muscovite. In the presence of 3 monominerallic clay fractions isolated from soils, the order of inhibition was: mica-beidellite > mica-vermiculite ≈ muscovite. In buffered suspensions all clay minerals depressed invertase activity to a generally greater extent after 24-hour than after 1-hour contact; no activity was detectable after

24 hours in the presence of montmorillonite. The inhibitory effect of pedogenic clay fractions were, hower, similar after 1- and 24-hour contact.

I.1.1.2. *Cellulase and hemicellulase.* According to the experiments described in detail by Lynch and Cotnoir [74], montmorillonite inhibited the activity of both cellulase and hemicellulase, while kaolinite inhibited only cellulase activity, namely when methylcellulose, and not hydroxyethylcellulose, was used as substrate. Lynch *et al.* [75] observed that, unlike montmorillonite, cellulase was not inactivated by attapulgite (substrate: methylcellulose). Contrary results were reported by Aomine and Kobayashi [8]: the activity of cellulase from *Aspergillus niger* (substrate: Na carboxymethylcellulose) was inhibited by allophane, but practically not by montmorillonite and halloysite.

A solution of cellulase from *Oxysporus* sp. was added to clay minerals; the enzyme was allowed to sorb for 16 hours and then the hydrolytic reaction was started by substrate (cellulose) addition. It was found (Pflug [104]) that cellulase activity was lowered by montmorillonite and to a lesser extent by palygorskite (a fibrous clay), while kaolinite had no effect. As a result of cellulase—montmorillonite complex formation, the pH optimum of enzyme shifted from 4.8 to 6.

I.1.1.3. *Amylases.* Lynch and Cotnoir [74] mentioned that montmorillonite and kaolinite did not inhibit amylase activity. Contrarily, Aomine and Kobayashi [8, 9] found that activity of β-amylase from barley or sweet potato was inhibited by clay minerals in the order: montmorillonite > allophane > halloysite, and the inhibition increased with the increase of clay concentration. With each clay, the inhibition was stronger when the enzyme, and not the substrate (soluble starch), was added first to the clay. The enzyme adsorbed to montmorillonite, allophane and halloysite retained 5, 15 and 30%, respectively, from the activity of free enzyme.

It was also found (Aomine and Kobayashi [8]; Kobayashi and Aomine [64]) that bacterial α-amylase and barley β-amylase behaved differently in respect of their inhibition by montmorillonite and allophane. In the case of α-amylase, allophane was the stronger inhibitor. Both clay minerals adsorbed the α-amylase, reducing the Michaelis constant (K_m) and maximum reaction velocity (V_{max}). The clays seemed to accelerate the formation of enzyme-substrate (ES) complex by adsorbing the enzyme and/or substrate on the external surface and, at the same time, to reduce the velocity of breakdown of the ES complex owing to modification of the configuration of enzyme molecule by adsorption. The adsorption of β-amylase to allophane resulted in an increase in K_m and a decrease in V_{max}. For β-amylase, allophane acted as a partially competitive inhibitor and concurrently as a partially noncompetitive inhibitor. Allophane was capable of partially inhibiting the formation of ES complex and also of modifying the configuration of the enzyme molecule.

According to Kobus [66], montmorillonite had a negligibly low inhibitory effect on a mixture of α- and β-amylases, while Velika-

nov *et al.* [122] found that the activity of these enzymes was more markedly inhibited by montmorillonite than by kaolinite. Activation energy of the amylolytic reaction increased following adsorption of enzymes. Bazelyan and Shafazhinskaya [14] reported a 45% inhibition of β-amylase activity by bentonite.

During 10-minute incubations, activity of α-amylase from *Bacillus subtilis* was strongly inhibited by palygorskite. Montmorillonite and kaolinite caused only a slight inhibition. It was deduced from kinetic data (K_m and V_{max}) that the inhibition brought about by palygorskite was non-competitive. Binding of enzyme to the clay was irreversible. The spontaneous inactivation of α-amylase, when its solution was kept at 25°C for 16 hours, was not prevented by any of the 3 clay minerals studied (Pflug [104]).

α-Amylase from *Bac. subtilis* and β-amylase from barley, following their addition to buffered suspensions of clay minerals or pedogenic clay fractions, behaved very differently. The clay minerals markedly depressed α-amylase activity after a contact period of 1 hour (order of the inhibitory effect: kaolinite ≈ montmorillonite > illite > allophane; muscovite had practically no effect). α-Amylase activity was strongly inhibited also by the 3 pedogenic clay fractions used. The effects of clay minerals and clay fractions on β-amylase activity were very marked, with activity barely detectable in all but the allophane-containing mixture (Ross [109]).

I.1.1.4. *Amyloglucosidase.* Amyloglucosidase (glucoamylase) adsorbed to clay showed less activity than did free enzyme (Usami *et al.* [121]). Amyloglucosidase from *Aspergillus niger* was inhibited by clay minerals in the order: palygorskite ⟩⟩ kaolinite » montmorillonite. In other words, this starch-cleaving enzyme behaved like α-amylase from *Bac. subtilis.* However, the K_m and V_{max} values of amyloglucosidase indicated that its inhibition by palygorskite was competitive and hence reversible. A 50% spontaneous inactivation occurred when the enzyme solution was kept at 25°C for 16 hours. The inactivation became complete in the presence of each clay (Pflug [104]).

I.1.1.5. *Lysozyme.* According to Ermol'eva and Buyanovskaya [38], the kaolinite-adsorbed egg white lysozyme was inactive against *Micrococcus lysodeikticus* cells.

I.1.1.6. *Chitinase.* Upon addition of chitin to kaolinite a chitin-kaolinite complex was formed, which reduced the activity of *Streptomyces* chitinase added. When chitinase was added to kaolinite before the chitin, a far more severe loss of activity occurred. Activity reached an intermediate value if chitinase was added to one half of the kaolinite and chitin to the other half of kaolinite and both complexes were mixed. The activities relative to control (chitin + chitinase) taken as 100% were 15.5, 5.5 and 10.6%, respectively. Upon adsorption of chitinase on kaolinite, the optimum pH shifted from 4.7 to 5.7 (Skujins *et al.* [114]).

I.1.1.7. *Catalase**. Liver catalase adsorbed to kaolin at pH 5 retained its activity and the adsorbed amount of enzyme could be eluted completely with phosphate buffer (pH 7.6) (Hennichs [52]). Catalase, like invertase and amylase, was more strongly inhibited by montmorillonitic clays than by kaolinite and monothermite (Zvyagintsev and coworkers [2, 122, 123, 127]). The optimum pH of the free catalase was found to be 6.95, but the activity of the enzyme adsorbed to Na-bentonite and Na-kaolinite was highest at pH 7.95 and 7.6, respectively (Aliev *et al.* [1]). In other studies (Stotzky [117]; Stotzky and Burns [118]), the activity of bound catalase on some homoionic montmorillonites was at least 4 times greater than that of free catalase, which suggested that binding altered the shape of the catalase molecule so that more active centres were exposed.

I.1.1.8. *Xanthine oxidase*. Adsorption of xanthine oxidase from milk to kaolin at pH 5 did not inactivate the enzyme, as 50—60% of the adsorbed enzyme amount was elutable with 1% Na_2CO_3 solution and the eluted enzyme was catalytically active (Dixon and Kodama [32]).

I.1.1.9. *Urate oxidase*. *Durand* [33, 34] found that both urate oxidase (uricase) and its substrate (Na urate) were adsorbed to bentonite. In adsorbed state, the enzyme remained active, but the activity diminished. The adsorbed substrate was not attacked, but the enzyme was able to replace and release it into solution. The free substrate was then oxidized by the adsorbed uricase.

I.1.1.10. *Glucose oxidase*. Montmorillonitic clays were stronger inhibitors of glucose oxidase activity than kaolinite and monothermite [123, 128, 129]. Glucose oxidase from *Penicillium notatum* was adsorbed completely by montmorillonite, allophane, illite in unbuffered and buffered (pH 5.6) suspensions but less strongly by kaolinite in a buffered suspension. Only montmorillonite and, to a lesser extent, allophane, however, significantly inhibited the activity (Ross and McNeilly [110]). But according to Morgan and Corke [92], the activity of *Aspergillus niger* glucose oxidase adsorbed either to montmorillonite or kaolinite was reduced markedly and remained low until almost the complete saturation of clay by adsorbed enzyme. Beyond that point, adsorption of small increments of enzyme resulted in large increases of activity, which approached that of free enzyme at the same pH. The adsorption of glucose oxidase involved a temperature-independent cation-exchange mechanism, and enzyme adsorbed to surfaces of clay could be desorbed in active form by elevation of pH of suspending solution. This was followed by a slower temperature-dependent fixation, probably, by hydrogen bonding, which resulted in protein being irreversibly adsorbed to clay surfaces and in loss of activity. On adsorption of glucose oxidase to montmorillonite surfaces, a conformational change of protein structure occurred, which allowed penetration of protein into the interlamellar spaces of this clay mineral. Morgan and Corke [93] also stated

* Conventionally, catalase and the other oxidoreductases are dealt with here as a group added to the enzymes of the carbon cycle.

that adsorption of the holoenzyme of glucose oxidase to montmorillonite or kaolinite at pH 4.5 was accompanied by release of the coenzyme (FAD). When the enzyme—clay complex structured ad pH 4.5 was adjusted to pH 6.5, data on desorption of both protein and FAD allowed the conclusion that the coenzyme desorbed as an integral part of the enzyme.

I.1.2. *Enzymes of the nitrogen cycle*

I.1.2.1. *Proteinases.* McLaren [86, 87] and McLaren and Estermann [89] studied the efect of kaolinite on the proteolytic enzymes chymotrypsin, trypsin and acetyltrypsin. Heat-inactivated lysozyme was used as substrate. In some experiments, first the enzyme and then the substrate were adsorbed on kaolinite. In other experiments, the adsorption was carried out in a reverse order. Proteolysis was slow when the enzyme was the first adsorbate. In other words, adsorption of enzyme on kaolinite brought about an inhibition of activity but did not cause the denaturation of enzyme. Adsorption of proteins was attributed partly to an ion-exchange mechanism and partly to simple physical adsorption at the external surface of the clay particle. Adsorption of a protein to kaolinite above the isoelectric point of the protein is relatively low or nil compared with the amounts adsorbed below the isoelectric point.

As the pH at kaolinite surface is lower by about 2 units than that of the bulk solution, a comparison of the activity of chymotrypsin on heat-inactivated lysozyme in solution and adsorbed on kaolinite has revealed that the pH optimum for maximum activity is narrower and shifted to a higher pH with adsorbed substrate (McLaren [83]; McLaren and Estermann [90]). Chymotrypsin hydrolyzed, in 24 hours, about 90% of the heat-inactivated lysozyme adsorbed on kaolinite and only about 70% of the monolayer of heat-inactivated lysozyme adsorbed to bentonite. The initial rate of hydrolysis of the bentonite—heated lysozyme complex was slower than that of the kaolinite—heated lysozyme complex. A purified proteinase from *Bac. subtilis* attacked the kaolinite—heated lysozyme complex at a rate almost identical to that by chymotrypsin (Estermann *et al.* [39]).

Aomine and Kokayashi [7, 8] demonstrated the inhibitory effect of 3 clays on "pronase-P" (a proteinase preparation from *Streptomyces griseus)* by using hemoglobin as enzyme substrate. The effect was stronger with allophane and montmorillonite than with halloysite. A part of the enzyme adsorbed on clays was released with Na acetate, and the enzyme which was not replaced with the acetate retained one fourth (allophane), a half (montmorillonite) or the whole (halloysite) of the activity of free enzyme. In another study, Kobayashi and Aomine [64] used both hemoglobin and a synthetic dipeptide (carbobenzoxy-L-glutamyl-L-tyrosine) as substrates for the *Str. griseus* proteinase, and found that montmorillonite was a stronger inhibitor than allophane. The

clays adsorbed the proteinase, reducing both K_m and V_{max}, *i.e.* the proteinase behaved like α-amylase (see Section I.1.1.3).

Adsorption of gelatinase (a papain-like bacterial proteinase) to H-, Na- and K-bentonites brought about activity inhibitions of 70, 60 and and 38%, respectively. Under the influence of the same clays, activity of caseinase (a trypsin-like bacterial enzyme) was inhibited to a lesser extent (60, 55 and 30%, respectively).A part (10—30%) of both enzymes could be desorbed with 5% solutions of ammonium salts, 10% Na acetate or 0.5% NaOH (Ambrož [3]). Solutions of alkaline and neutral proteineses (pH optima: 8.5 and 6.5, respectively) from *Serratia marcescens* and *Bacillus megaterium* were treated with 5% montmorillonite, illite or kaolinite. In each case, montmorillonite was the strongest and kaolinite the weakest inhibitor. The enzymes of *S. marcescens* were more sensitive to clay-provoked inhibition than those of *Bac. megaterium,* and alkaline proteinases of both bacteria were more sensitive than their neutral proteinases (Ambrož]5, 6[).

Montmorillonite inhibition strongly the activity of trypsin and somewhat less that of the pepsin. The degree of inhibition increased with increasing clay amount (Kobus [65, 66]). Pronase adsorbed on bentonite and kaolinite, then treated with enzyme substrate (casein) lost 55.6 and 46.3%, respectively, from the activity of free enzyme (Aliev and Zvyagintsev [2]).

Activity of the SH-dependent proteinase, papain, adsorbed on kaolinite was 30% less than that found with papain in solution. α-N-Benzoyl-L-arginine ethyl ester was used as substrate and dithiothreitol served as reducing agent. Similar experiments were performed with soluble and adsorbed papain in the presence of several mixtures of an oxidizing disulphide and a reducing thiol in various molar ratios. When both disulphide and thiol were uncharged, neutral compounds (dithiodiglycol and β-mercaptoethanol, respectively), no difference in relative activity as a function of the ratio of these compounds was observed with soluble or adsorbed papain. By contrast, the relative activity of papain — as compared to that of soluble papain — was considerably less in mixtures of dithiodiglycol and β-aminoethyl mercaptan (+1 charged thiol). Similar results were obtained with cystine dimethyl ester (+2 charged disulphide) and cysteine ethyl ester (+1 charged thiol) (Benesi and McLaren [15]).

I.1.2.2. *Peptidases.* Haskå [50] has shown that kaolinite- and montmorillonite-adsorbed bacteriolytic (cell wall lytic) enzymes from cell-free culture solution of *Myxococcus virescens* could lyze *Micrococcus lysodeikticus* cells. One of the bacteriolytic enzymes (alanyl-ε-N-lysine endopeptidase) was desorbable as active enzyme. This means that adsorption did not inactivate at least one of the enzymes.

I.1.2.3. *Urease.* According to the observations by Pinck and Allison [106] and Pinck [105], urease was adsorbed completely by H-montmorillonite whereas incomplete immobilization occurred with untreated montmorillonite and H-kaolinite. Comparative activities of the various urease complexes, based on the activity of urease in solution

as 100, were approximately as follows: H-montmorillonite 25, H-kaolinite 50, and untreated montmorillonite 66. The adsorbed urease, in the presence of urea, was gradually released from the clay and exerted its activity in solution. Initial release of the enzyme from the clay was attributed to urea acting as a cation. Subsequently, the ammonia evolved from the hydrolysis of urea became the active cation. But in Durand's [35, 36] experiments, ureolysis catalyzed by the urease—bentonite complex did not require elution of the enzyme. Neither urea nor ammonium ions were able to separate urease from the complex. A pH increase only induced the release of urease into the solution. The activities were much lower with urease—bentonite complex than with free urease, when both activities were measured at the same pH and substrate concentration. The optimum substrate concentration was higher for the complexed urease than for the free one. Similarly, the pH optima were 7.7 and 7.1, respectively.

Activity of the urease extracted from bean flour decreased nearly proportionately to the amount of montmorillonite added (Kobus [66]). But in the experiment of Burns *et al.* [26], initially, adsorption of jackbean urease onto bentonite increased its activity above that shown by the urease alone. At the same time, the activity of a urease solution decreased greatly within a few minutes after mixing the solution with montmorillonite (Lampe and Aldag [73]).

Makboul and Ottow [76] found that inactivation of jackbean urease adsorbed to Ca-montmorillonite or Ca-kaolinite was significantly retarded when, instead of water, 0.05 M THAM buffer solution (pH 9) was added to the reaction mixtures. It was also established (Makboul and Ottow [77]) that activity of jackbean urease was reduced by increasing amounts of various clay minerals in the following order: montmorillonite > bentonite > illite > kaolinite > halloysite > pyrophyllite.

Activity, K_m and V_{max} values of the free jackbean urease significantly differed from those obtained when the enzyme was added to Ca-homoionic montmorillonite, kaolinite or illite. In the presence of clays, the enzyme activity decreased. The highest decrease (77—80%) was recorded at the highest clay concentration used and lowest substrate concentration tested (with montmorillonite and kaolinite) or a relatively low substrate concentration (with illite). The K_m values increased greatly in the enzyme-montmorillonite mixtures and to a lesser extent in those with kaolinite and illite, and with each clay the increase was proportionate to the clay concentration. The V_{max} values decreased with increasing amounts of kaolinite and illite. But in the case of montmorillonite, the highest value of V_{max} was obtained at the highest clay concentration. This suggests that a definite amount of montmorillonite at a certain set of suitable conditions may even increase the V_{max} (Makboul and Ottow [78]).

I.1.3. *Enzymes of the phosphorus cycle*

I.1.3.1. *Ribonuclease.* Bower [20] was the first to observe that the activity of nuclease in solution was reduced by the presence of bentonite. Many investigators (*e.g.* Brownhill *et al.* [22]; Click and Hackett [28]) used bentonite to efficiently inhibit RNase activity which is necessary during isolation of RNA from viruses, microbial, plant and animal cells. Inhibition of pancreatic RNase by bentonite was attributed (Jacoli [56]; Jacoli *et al.* [57] to a combination of two mechanisms: ion exchange and masking of the active site or simply a distortion of the enzyme molecule in the process of entering the interlayer spaces of the clay mineral. Vermiculite has a much higher cation-exchange capacity and a more limited expansion of interlayer spaces than bentonite. Thus, vermiculite does not make possible for RNase to enter the interlayer spaces and, consequently, it does not inhibit RNase activity. According to Aseeva and Panikov [10], the clay minerals that strongly inhibited RNase activity comprised not only bentonite, kaolinite and muscovite, but vermiculite, too. The enzyme was extractable from clays only to a less extent. In the experiment of Marty and Bastide [84], montmorillonite inhibited completely activity of the pancreatic RNase. The enzyme was strongly adsorbed; it was not eliberated by washing with diluted NaCl solution.

I.1.3.2. *Other phosphatases.* Mortland and Gieseking [94] demonstrated the inhibitory effect of clays on the activity of phosphatases hydrolyzing phytin, fructose diphosphate, glycerophosphate and lecithin, respectively. The enzyme sources were: wheat bran (phytase), baker's yeast (fructose diphosphatase and glycerophosphatase) and dried kidney cortex (lecithinase). The degree of inhibition varied with the kind of clays according to the following order: Wyoming bentonite (montmorillonite) > Putnam-like clay > illite > kaolinite, and was roughly proportional to the base-exchange capacity of clays. This indicated that phosphatases were adsorbed on clays, at least partially, as cations. The relationship between the inhibitory effect of clays on phytase activity and their amount was linear.

Ramirez-Martínez and McLaren [107] found that the activity of wheat acid phosphatase adsorbed on kaolinite was reduced by approximately 75% when compared with that of the enzyme in solution. pH optimum was 5.7 for the enzyme in adsorbed state and 5 for the enzyme in solution.

Pacha and Rait [101] studied the adsorption of alkaline phosphatase (from *Escherichia coli)* on kaolinite and bentonite at pH 6—8.5. At pH < 7, both clays adsorbed the enzyme irreversibly. Bentonite adsorbed more enzyme than kaolinite. At pH 8, only kaolinite was able to adsorb the enzyme, irreversibly. In adsorbed state at pH 6, the enzyme was catalytically inactive. At pH 8, the enzyme was active, but its specific activity decreased with increasing saturation of clay surface by the enzyme molecules. K_m was about 5 times higher with the adsorbed enzyme than with the free one.

Makboul and Ottow [77] established that pyrophyllite, which had the weakest inhibitory effect on urease activity (see Section I.1.2.3), manifested the strongest inhibition on the activity of acid phosphatase from wheat. The other clay minerals studied inhibited this activity in the order: montmorillonite > bentonite > halloysite > kaolinite > illite. Makboul and Ottow [79, 81] also found that the addition of increasing amounts of Ca-homoionic montmorillonite *(M)*, kaolinite *(K)* and illite *(I)* decreased the activity of acid phosphatase at each aubstrate level tested and increased the K_m value, in the order: $M \gg K > I$. The activity decrease expressed as % of the activity of free enzyme diminished with increasing substrate concentration and grew with rising amounts of clays. The greatest activity decreases were of 98, 92 and 89%, respectively. The V_{max} remained nearly constant at different amounts of *K* and *I*, but increased remarkably with rising concentrations of *M*.

In another experiment (Makboul and Ottow [80, 81]), a pure alkaline phosphatase (AP) of intestinal origin was studied. The same Ca-homoionic clays were used as in the experiment with acid phosphatase (see the preceding paragraph). The results were also similar, but with some differences. The clays decreased AP activity in the order: $I > M > K$; at highest clay and lowest substrate concentration the decreases were of 64, 37 and 22%, respectively. *I* decreased, *M* and *K* increased the value of K_m, as compared to that of the free enzyme. V_{max} was diminished by *I* and *K* and became enhanced in the presence of *M*. The aberrant behaviour of *I*, namely the decreased K_m, *i.e.* the increased enzyme—substrate (ES) complex formation in the presence of *I*, in combination with a lowered hydrolysis of the sorbed ES complex, was explained by a modification of the AP configuration as a consequence of sorption. Such a modification in the enzyme configuration could have facilitated the formation of ES complex but may have increased its stability against subsequent hydrolysis. One can also hypothesize that the aberrant behaviour of *I* is related to the catalytic action of potassium ions of this clay.

The effect of 5 pedogenic clays of different composition (containtive type in the case of acid phosphatase (Makboul and Ottow [79]). Alkaline phosphatase is also inhibited competitively by Ca-homoionic *K* and *M*, but uncompetitively by Ca-homoionic *I* (Makboul and Ottow [80, 82]).

The effect of 5 pedogenic clays of different composition (containing at least two clay minerals homoionic to Ca) on the activity, K_m and V_{max} of acid and alkaline phosphatases was also studied (Ottow *et al.* [100]). Each clay inhibited activity of both enzymes. In general, V_{max} decreased with increasing immobilization of enzymes. K_m remained constant or increased considerably depending on the composition of clays. The changes in V_{max} and K_m were both clay and enzyme specific. In the case of 4 clays, immobilization of acid phosphatase was reflected by a competitive type of inhibition, while that of alkaline phos-

phatase resulted in a mixed type of inhibition. Non-competitive inhibition was recorded with acid phosphatase in the presence of a predominantly *M* clay and with alkaline phosphatase after adsorption to mixed and interlayered clays of *I-M*-chlorite type.

The alkaline phosphatase (AP) was desorbed from Ca-homoionic clay minerals by extraction with Tris-malate-citrate buffer (Makboul and Ottow [83]). In the case of *M* and *K*, the V_{max} recorded for the enzyme in extract was higher than that for the non-desorbed enzyme present in the sediment. The opposite was true in the case of *I*. AP desorbed from or remained sorbed to *M* had the same K_m value, while the enzyme in the extracts from *K* and *I* was characterized by higher K_m values in comparison with those found for the enzyme of the sediment. The increase in K_m of the desorbed AP as well as its decreased V_{max} may be ascribed to a decrease in substrate affinity by a part of AP which may be still intimately associated with some clay components or has suffered some conformational changes.

Dick *et al.* [31] found that sorption of maize-root acid phosphatase by illite, kaolinite and montmorillonite decreased the activity significantly, but it did not eliminate the activity entirely.

I.1.4. *Enzymes of the sulphur cycle*

I.1.4.1. *Arylsulphatase.* The affinity between kaolinite and arylsulphatase was found (Simpson and Hughes [112]; Hughes and Simpson [53]) to decrease with increasing solution enzyme concentration. At the same time, the montmorillonite—arylsulphatase affinity increased up to a solution enzyme concentration of about 15 mg/100 mg clay, and decreased at higher enzyme concentrations Both clay minerals reduced arylsulphatase activity. The reduction was stronger with montmorillonite than with kaolinite and was related to the total surface area of clay available for adsorption. Adsorption of arylsulphatase by kaolinite was confined to external surfaces, but with montmorillonite interlattice sites were also involved. Arylsulphatase held as a monolayer on exterior surfaces ('primary surface' adsorption) as well as that intercalated by montmorillonite lost its activity. The remainder ('secondary surface' adsorption) retained its activity and could be removed from the clay—enzyme complex by elution.

I.2. Activity of Enzymes Added to Soil Samples

I.2.1. *Enzymes of the carbon cycle*

I.2.1.1. *Invertase.* The invertase activity of yeast autolysate added to samples of a leached chernozem suffered only a slight diminution (Kiss [62]). At the same time, the activity of yeast invertase-soil mixtures was inhibited by Pb^{2+} aniline and *p*-toluidine to a lesser ex-

tent than that of the free enzyme (Kiss [60]). But the yeast invertase added to soil samples previously heated at 105°C for 3—4 hours lost a significant part of its activity. The effect of two chernozems was stronger than that of a soddy podzol (Zvyagintsev and Velikanov [128, 129]). The invertase added to soil samples remained active, but, unlike catalase (see Section I.2.1.3.), was inhibited by tannic acid to the same extent as the solution enzyme (Kunze and Rickart [72]). In the experiment of Ross [109], the two topsoils to which yeast invertase was added did not sorb the enzyme or inhibit its activity.

I.2.1.2. *Amylases.* An α- and β-amylase-containing preparation from barley malt was added to samples of leached chernozem, calcareous chernozem, chestnut soil and solonchak. The enzyme activity decreased immediately in the solonchak, but suffered no immediate change in the other soils (Galstyan [41, 42]). The two topsoils studied by Ross [109] had no significant influence on the activity of added bacterial α-amylase or barley β-amylase.

* * *

I.2.1.3. *Catalase.* Samples of a brown soil were heated at 105°C for inactivation of their catalase content. These samples were then treated with pure catalase and an inhibitor (tannic, gallic or *p*-hydroxybenzoic acid). Mixtures without soil and/or inhibitor served for comparison. It was found that in the presence of soil the inhibition of catalase activity was greatly diminished (Kunze [70]; Gnittke and Kunze [44]). The inactivation of enzyme in a buffer solution was also diminished by the heat-inactivated soil when the humidity in mixtures exceeded the maximum water-holding capacity of soil. At lower humidities, the soil caused a great loss in the activity of added catalase (Kunze [71]).

A bacterial catalase, following its adsorption to a clayey soil, retained most of its activity. The adsorbed enzyme remained stable: during 14 days the activity loss was only of 7.17% (Cervelli and Aringhieri [27]). But in other experiments, up to 50% losses occurred in the activity of catalase following its addition to samples of 5 soils (Aliev and Zvyagintsev [2]). The losses were even higher in the 5 soils studied when the catalase-soil mixtures were submitted to air-drying before activity determination (Zvyagintsev and Aliev [127]). The activity losses in samples of 4 soils ranged between 70 and 85%, and were only of 60—70% when the catalase was adsorbed to autoclaved samples of the same soil (Tul'skaya and Zvyagintsev [119]).

I.2.1.4. *Peroxidase.* Activity of horseradish peroxidase, after its addition to samples of a solonchak, immediately decreased. The other soils studied (leached chernozem, calcareous chernozem and chestnut soil) did not affect immediately the activity of the added peroxidase (Galstyan [41, 42]).

I.2.1.5. *Glucose oxidase.* Heated soil samples added to glucose oxidase solution caused significant reductions in the enzymatic activity. This effect was stronger with two chernozems than with a soddy podzol (Zvyagintsev and Velikanov [128, 129]). Ross and McNeilly [110] found that the effect of soils on the activity of added glucose oxidase (from *Penicillium notatum*) differed considerably in unbuffered and buffered (pH 5.6) suspensions. Glucose oxidase was less active and more strongly adsorbed in unbuffered suspensions of moderately acidic soils than of nearly neutral soils. Activity could be influenced by the type of clay present; however, the effect of montmorillonite in soil was evident only in a buffered system.

I.2.2. *Enzymes of the nitrogen cycle*

I.2.2.1. *Proteinases.* Pronase added to the silt-clay fraction of a clay-loam soil retained its hydrolytic capacity on α-N-benzoyl-L-arginine amide during incubation (1—192 hours) (Burns *et al.* [25, 26]). When added to intact soil samples, pronase retained 60—68.2% of its initial activity on 7-day incubations (Pettit *et al.* [102, 103]). Other experiments also indicated that pronase (Aliev and Zvyagintsev [2]; Zantua and Bremner [126]; Vorob'eva and Zvyagintsev [124]; Mas'ko *et al.* [85]) and trypsin [126] remained active for at least one day after their addition to samples of different soils. Shih and Souza [111] incubated pronase-treated samples of a sandy loam for 90 minutes and found that the enzyme remained active.

I.2.2.2. *Urease.* In some alluvial soils urea used as a fertilizer had only a weak effect on the yield of potatoes, a fact ascribed to the poor ureolytic capacity of these soils. When such a soil was treated with soybean urease, the rate of ureolysis and the crop yield increased considerably (Bordas and Mathieu [19]). In Turchin's [120] experiments, soybean urease added to soils remained capable to hydrolyze urea, but it did not enhance the effect of urea on the yield of oats.

A fine sandy loam preheated to 85°C, then treated with jackbean urease and analyzed immediately showed high initial activity, but on incubation under unsterile conditions the activity rapidly diminished (Conrad [29]). The added urease was less stable in the presence of toluene than in its absence (Conrad [30]). When a solution of crystalline urease was added to autoclaved samples of two different soil types, the amount of urea hydrolyzed was not significantly different. Under sterile conditions, 50% or more of the activity of added urease was lost during the first 12 hours of contact with soil. The rate of decline was greater in a clay soil than in a fine sandy loam (Stojanovic [116]). Ammonia volatilization from a urea-treated silt loam soil, to which a urease preparation was also added, greatly increased during the first day of incubation at 28°C as compared to the soil treated only with urea (Moe [91]). One can deduce from this observation that the added urease retained at least a part of its activity.

Samples of each layer of the surface organic horizon of a black spruce *(Picea mariana)* stand were treated with urease, then tested for urease activity on 8-hour incubations. Added urease was active in all samples. Additional urease activity was greatest in unsterilized samples, less in those sterilized by γ-irradiation, least in steam-sterilized ones, and decreased with the depth of layers (R o b e r g e [108]). Mixtures were prepared from soybean urease and soil samples and immediately analyzed. The enzyme lost a part of its activity when the soil was a solonchak, and remained unchanged in a leached chernozem and a chestnut soil (G a l s t y a n [42]). Jackbean urease, immediately after its addition to unsterilized and sterilized (autoclaved) soil samples, resulted in a marked increase in urease activity, but this effect diminished rapidly with time and was in some soils insignificant after 1 or 3 days and in all soils negligible after 7 or 14 days. The rates of inactivation of added urease were faster with sterilized than with unsterilized samples (Z a n t u a and B r e m n e r [126]).

Only slight decreases occurred in urease activity at the moment of mixing enzymatically active soil and pure urease solution. Destruction of enzyme during 24—96-hour incubations should be attributed to the developing microorganisms and not to adsorption (B e r i and B r a r [16]; V o r o b ' e v a and Z v y a g i n t s e v [124]). B e r i *et al.* [17] studied the downward movement of surface-applied urea, with or without addition of urease, in columns with a sandy loam and a silty clay loam. Urease addition resulted in a temporary increase of activity, *i.e.* conversion of more urea-N into NH_4^+-N which was retained in the upper part of soil columns. The urease-treated sandy loam (0—28 cm depth) conserved 95%, while the untreated soil retained 23% of the urea-N applied. In the 0—16 cm depth of the silty clay loam column the corresponding values were 91 and 54%, respectively. The authors have drawn the conclusion that by artificially increasing soil urease activity it is possible to minimize the leaching losses of urea. S i n g h a l and K u m a r [113] consider that urease—boron mixtures applied in soil induce photosensitization in plants so as to accelerate the overall rate of their synthetic processes.

A mixture was prepared from urease solution and a sterilized soil sample (loamy riverside soil) and immediately analyzed to determine its enzyme activity in comparison with that of urease solution. Similar values were registered (L a m p e and A l d a g [73]).

I.2.2.3. *Asparaginase.* The diminution in activity of yeast asparaginase due to its adsorption to soil samples was of 37% (in a marsh soil; pH 5.6) and 0.7% (in a garden soil; pH 4.6) (M o u r a r e t [95]).

I.2.3. *Enzymes of the phosphorus cycle*

I.2.3.1. *Phytase.* J a c k m a n and B l a c k [54, 55] demonstrated that the phytase extracted from wheat bran and then added to soil samples retained completely its activity. The activity decreased only in soil—

phytase mixtures submitted to air-dryng or continuous shaking. The decrease was ascribed to adsorption of enzyme to soil particles which resulted in its inhibition.

I.2.3.2. *Deoxyribonuclease.* With genetically labelled strains of *Bacillus subtilis* growing together in autoclave-sterilized soil, high frequencies of transformation were obtained, even when bovine pancreatic DNase was added to the soil. The inability of DNase to stop transformation in soil culture, in contrast to its ability to do so in laboratory culture, was attributed to its adsorption and inactivation by soil particles (Graham and Istock [47]).

I.2.3.3. *Acid phosphatase and inorganic pyrophosphatase.* When 10 mg of maize-root homogenate was mixed with 1-g soil samples, the inhibition of acid phosphatase and inorganic pyrophosphatase activities of homogenate by 12 soils ranged from 43 to 63% (average = 52%) and from 11 to 62% (average = 44%), respectively. The inhibition of the activity of purified acid phosphatase from wheat germ ranged from 88 to 95% (average = 92%). The degree of inhibition of acid phosphatase and pyrophosphatase by steam-sterilized soils was less than by unsterilized soils (Dick *et al.* [31]).

I.2.4. *Enzymes of the sulphur cycle.* No literature data are available.

I.3. Activity of Soil Enzymes in Clay Mineral—Soil Mixtures

I.3.1. *Enzymes of the carbon cycle*

I.3.1.1. *Invertase.* Invertase activity in samples of a leached chernozem was not influenced by 5—50% kaolinite or 5—25% bentonite additions (Kiss [62]). In other words, the invertase already accumulated in this soil could not be inhibited by pure clay minerals. But in the case of another soil, studied by Kozlov *et al.* [67], 1—10% bentonite additions led to 26—100% inhibitions of invertase activity.

I.3.1.2. *Cellulase.* The soil treated by Kozlov *et al.* [67] with 1—10% bentonite lost 31—83% of its initial cellulase activity. In another experiment, samples of a soddy soil were amended with 8% kaolinite or bentonite. Kaolinite caused a slight reduction, while bentonite exhibited a complete depression of soil cellulase activity (Nováková and Šiša [97]).

Badura *et al.* [12, 13] carried out pot experiments using a beech forest soil (pH 4.6) enriched with 10 or 20% bentonite, moistened to about 30% humidity and incubated for 42 days. Cellulase activity was always lower in the bentonite-treated soil than in the control (no bentonite addition).

I.3.1.3. *Amylase.* In B a d u r a and co-workers' pot experiments mentioned above, amylase behaved like cellulase.

* * *

I.3.1.4. *Catalase, peroxidase and diphenol oxidase.* The 10 and 20% bentonite additions in the experiment of B a d u r a *et al.* [12, 13] brought about little changes in catalase activity and considerable diminutions in the activity of the other two enzymes.

I.3.1.5. *Dehydrogenases.* Mixtures prepared from 10-g samples of a garden soil and 0.1—0.8 g clay mineral in untreated and Ca-homoionic forms were submitted to dehydrogenase activity determination, *i.e.* to determination of electron transport system activity in microbial cells. Soil without added clay served as control. Clay additions resulted in an activity decrease, the degree of which was proportionate to the amount of clays and depended on their nature. For example, in the case of 0.6 g clay addition the following decreases were registered (activity of control = 100%): montmorillonite 87.8, bentonite 67.4, kaolinite 37.1, illite 20.0, pyrophyllite 4.0, and halloysite 2.4%. The untreated and Ca-homoionic clays behaved similarly (M a k b o u l and O t t o w [77]).

Enrichment of soil with 10% and especially with 20% bentonite resulted in a great loss in dehydrogenase activity (B a d u r a *et al.* [12]).

I.3.2. *Enzymes of the nitrogen cycle*

I.3.2.1. *Proteinases and urease.* In a forest soil to which 5% bentonite was added, then moistened to about 40% humidity and incubated for 28 days, proteinase and urease activities were lower than in the untreated soil (B a d u r a *et al.* [11]). Proteinase activity was also determined in an experiment in which 10 or 20% bentonite was added to the forest soil and it was found to be lower than in the control soil [13].

I.3.3. *Enzymes of the phosphorus cycle*

I.3.3.1. *Phosphomonoesterases.* Mixing samples of 3 soils (sandy, loamy and clayey) with montmorillonite or kaolinite did not bring about any changes in the soil phosphatase activity measured in unbuffered reaction mixtures (K r o l l and K r á m e r [68]; K r o l l *et. al* [69]). But the alkaline phosphatase activity in samples of a weakly leached chernozem, to which montmorillonite was mixed, decreased in parallel with the amount of added clay (G o i a n [45, 46]).

Both acid and alkaline phosphatase activities decreased in a forest soil enriched with 10 or 20% bentonite (B a d u r a *et al.* [13]).

I.3.4. *Enzymes of the sulphur cycle.* No literature data are available.

I.4. Activity of Enzymes Added to Clay Mineral—Soil Mixtures

I.4.1. *Enzymes of the carbon cycle*

I.4.1.1. *Invertase.* Activity of the yeast autolysate invertase added to mixtures of leached chernozem + kaolinite or bentonite remained unchanged in contrast to its strong inhibition which occurred when the enzyme was added to pure clay minerals. This means that the soil removed the inhibitory effect of pure clay minerals on enzyme activity (Kiss [61]).

I.4.2, I.4.3 and I.4.4. *Enzymes of the nitrogen, phosphorus and sulphur cycles.* No literature data are available.

II. Stabilizing and Protecting Effect of Clay Minerals on Enzymes

II.1. *Enzymes of the carbon cycle*

II.1.1. *Invertase.* Mixtures prepared from samples of a leached chernozem + 2—20% kaolinite or 2—10% bentonite were amended with enzyme substrate (sucrose) and moistened, then incubated for 3 weeks to induce the microbial synthesis of invertase. Variants not amended and not moistened as well as variants without added clay minerals were also included. The results showed that more invertase accumulated in the clay mineral—soil mixtures than in the soil samples. This proves that the clay minerals have stabilized and protected from decomposition the invertase molecules produced by soil microorganisms under the inducing action of sucrose. Practically no change occurred in the invertase activity of variants without sucrose (Kiss [59]).

In experiments for recultivation of sand open cast mine floor drifts, Hazuk [51] and Greszta and Olszowski [48] determined invertase and other enzyme activities in the recultivated sand. The recultivation experiments comprised many variants (each in 4 plots): controls (no fertilization); mineral fertilizers; humus (= material from the humus-mineral horizon mixed with forest leaf-mould) 60 t/ha + mineral fertilizers; sorbent-fertilizer 30 t/ha; sorbent-fertilizer 60 t/ha; sorbent-fertilizer 60 t/ha + peat dust 12 t/ha; cinders 60 t/ha + mineral fertilizers. The sorbent was produced from bentonite containing 73—74% clayey components (montmorillonite, kaolin, illite). The sorbent-fertilizer consisted of bentonite to which lime and mineral fertilizers were added. Following fertilization, a seed mixture of perennial plants, predominantly legumes, were sown in all plots. Invertase acti-

vity measured during two years was highest in the sand of the plots with sorbent-fertilizer or with sorbent-fertilizer + peat.

Olszowski [98] performed fertilization experiments in a pine *(Pinus silvestris)* forest. The variants were the following: control; NPK; bentonite 10 t/ha; bentonite 30 t/ha; CaO; NPK + bentonite 10 t/ha; NPK + bentonite 30 t/ha; NPK + CaO. During the first year after fertilization no change was produced in the invertase and other enzyme activities of the fertilized soils relative to the control soil. During the second and the third year, soil invertase activity increased in each fertilized variant. The degree of increase was similar in the variants treated with NPK or bentonite or NPK + bentonite, and reached the highest value in the NPK + CaO variant.

II.1.2. β-*Glucosidase*. In the sand recultivation experiments (Greszta and Olszowski [48]) and in the forest fertilization experiments (Olszowski [98, 99] (see Section II.1.1.), β-glucosidase behaved like invertase.

II.1.3. *Cellulase and hemicellulase*. Sörensen [115] prepared mixtures from a sandy soil, montmorillonite, hemicellulose or glucose and NH_4NO_3 and incubated them under humidity and temperature conditions favourable for the growth of microorganisms. During incubation, hemicellulase induction and accumulation took place in the hemicellulose-treated mixtures, and the enzyme activity was higher in the variants with added montmorillonite than in those without added clay. At the same time, hemicellulase activity remained practically unchanged in glucose-amended mixtures treated or not with montmorillonite.

In the experiment of Nováková and Šiša [97], addition of 8% kaolinite to samples of a soddy soil not amended or amended with 1% cellulose brought about an increase in cellulase activity during the 84-day incubation, as compared to the variant to which no clay mineral was added. Under similar conditions, bentonite addition resulted in lower cellulase activity.

II.1.4. *Amylases*. Residual activity of α- and β-amylases adsorbed to bentonite and kaolinite decreased significantly only at 60°C, while inactivation of free enzymes began at 40°C. The temperature coefficient (Q_{10}) in the 9—49°C zone was higher for the adsorbed than for the free enzymes (Velikanov *et al.* [122]). Nováková [96] amended samples of a soddy soil and soil + kaolinite or bentonite mixtures with starch and an ammonium salt. Variants without added starch were used as controls. During incubation (84 days), amylase activity increased in the starch-amended variants and especially in those amended with both starch and ammonium salt. The increase was lower in the clay—soil mixtures than in the soil.

II.1.5. *Amyloklucosidase*. Stabilities towards heating and pH treatment were lowered by adsorption of amyloglucosidase (glucoamylase) to clay. The adsorbed enzyme was inhibited by mercuric acetate far more markedly than the free enzyme (Usami *et al.* [121]).

I.1.6. *Catalase.* 'Catalytic activity' (H_2O_2-splitting by catalase and nonenzymatic factors) was measured on 5 soils after 10-day incubation with and without added bentonite and organic matter (glucose or ground alfalfa). The activity was affected by soil type, addition of bentonite and organic matter and drying of the semples. The two-factor interrelations of bentonite with soil type, organic addition and drying were highly significant and the three-factor interrelation of bentonite × soil type × organic addition was significant (Johnson and Temple [58]).

Catalase activity in the sand submitted to recultivation (see Section II.1.1) was highest in the variants treated with bentonite-containing sorbent-fertilizer especially when peat was also administered [48, 51]. In the forest fertilization experiments of Olszowski [98, 99] (see Section II.1.1), catalase activity varied in the same sense as invertase activity.

The Q_{10} value in the 9—49° zone was higher for the catalase adsorbed to bentonite or kaolinite than for the free enzyme. A 15-minute heat treatment at 70°C left unchanged the catalase adsorbed to H-bentonite and destroyed most of the free catalase. At 100°C both adsorbed and free catalases were completely inactivated (Velikanov *et al.* [122]). In the presence of bentonite or kaolinite, catalase was more resistant to pronase than the free enzyme (Aliev and Zvyagintsev [2]). To explain the inability of bacterial proteolytic enzymes to degrade catalase bound to some homoionic montmorillonites, it was suggested (Stotzky and Burns [118]) that binding altered the shape of the enzyme molecule so that the sites for attachment and/or action by proteolytic enzymes were masked.

II.1.7. *Glucose oxidase. Aspergillus niger* glucose oxidase adsorbed to montmorillonite was found (Morgan and Corke [92]) to be heat-labile: it lost 96% of its activity after 5 minutes at 60°C as compared to a loss of only 15% for the free enzyme.

II.1.8. *Dehydrogenases, polyphenol oxidase and peroxidase.* The changes occurring in the soil dehydrogenase activity of the fertilized forest plots (see Section II.1.1) showed the same pattern as invertase activity [98, 99]. In peatland reclamation experiments (Gavrilkina [43]), montmorillonite application at a rate of 200 m^3/ha led to increases in dehydrogenase and polyphenol oxidase activities and to a decrease in peroxidase activity of the peat.

II.2. *Enzymes of the nitrogen cycle*

II.2.1. *Proteinases.* Ensminger and Gieseking [37] found that proteins, when adsorbed onto montmorillonite, were in large measure resistant to hydrolysis by proteolytic enzymes (pepsin in acidic and pancreatin in basic solution). Kaolinite, due to its low base-exchange capacity, had no significant effect on proteolysis.

Chymotrypsin degraded to a lesser extent the heat-inactivated lysozyme adsorbed on kaolinite than the denatured lysozyme free in solution. Similarly, adsorbed trypsin was slowly hydrolyzed by free trypsin in a suspension of kaolinite. However, the protecting effect of adsorption was weak (McLaren [87]).

Bacterial gelatinase and caseinase adsorbed to bentonite, then heated for 45 minutes retained a considerable part of their activity, namely about 70 and 40%, respectively, at 100°C, and about 50 and 25%, respectively, at 120°C (Ambrož [3]).

Ambrož [4] incubated samples of a podzol with 0.5% gelatin, 0 or 15% bentonite for 3 months, during which the conditions for the development of microorganisms were favourable. After this incubation period, the highest gelatinase activity was recorded in the bentonite-treated soil. One can deduce from this observation that bentonite protected the newly synthesized enzyme molecules from their degradation.

A bentonite—jackbean urease complex, submitted to a 12-hour digestion with pronase, retained some of its activity (Burns *et al.* [25, 26]).

Griffith and Thomas [49] determined the activity of soluble and immobilized pronase in the presence of montmorillonite homoionic to Na, NH_4, Ca or bivalent Cu. This study presents much interest for our review, too, because the accumulated soil enzymes may be considered as immobilized enzymes and montmorillonite as an enzyme inhibitor. These investigators found that the pronase immobilized on carboxymethylcellulose was not subject to the same inhibition in the presence of clay as the soluble enzyme. On the basis of equivalent amounts of enzyme protein, the immobilized pronase had only 28% of the activity of the soluble enzyme in the absence of clay. In the presence of clay, the immobilized pronase retained an average of 75% of its normal activity, while the activity retained by the soluble pronase averaged only 31% of its normal activity.

II.2.2. *Urease*. Bentonite was found to protect urease against inhibitory concentrations of Cu^{2+}. The protecting effect was explained by the retention of Cu^{2+} on the clay. Alloxan, which does not bind to bentonite, inhibited both free and complexed urease, to the same extent (Durand [35, 36]).

The recultivated sand (see Section II.1.1) exhibited the highest urease activity in the plots to which bentonite-containing sorbent-fertilizer was applied alone or with peat [48, 51]. In the forest fertilization experiments of Olszowski [98, 99], the behaviour of urease was similar to that of invertase (see Section II.1.1).

Hydrolysis of urea by jackbean urease took place at —10 or —20°C in the presence, but not in the absence, of montmorillonite, illite, kaolinite or autoclaved (120°C/1 hour) soils. At —30°C neither clay minerals or autoclaved soils could protect the enzyme (Bremner and Zantua [21]).

The observation, that urease activity in a sterilized soil—urease mixture decreased to a lesser extent than in the enzyme solution, both being kept at room temperature for 6 days, was interpreted as evidence of the enzyme-stabilizing effect of the soil (L a m p e and A l d a g [73]).

II.2.3. *Asparaginase.* In sand recultivation experiments, application of bentonite-containing sorbent-fertilizer without or with peat brought about the highest increase in asparaginase activity of sand [48, 51]. In O l s z o w s k i ' s [98] forest fertilization experiments, asparaginase activity showed the same trend of changes as invertase activity.

II.3. *Enzymes of the phosphorus cycle*

II.3.1. *Alkaline phosphatase. E. coli* alkaline phosphatase adsorbed to bentonite or kaolinite lost most of its activity when submitted to a 10-minute heating at 40—60°C. Under the same conditions, the free enzyme was heat-stable (P a c h a and R a i t [101]).

II.4. *Enzymes of the sulphur cycle.* No literature data are available.

Discussion

Two problems will be discussed.

1. The literature data reviewed prove that *in general* the clay minerals inhibit the activity of enzymes which is followed by stabilization and protection of the residual activity. The contrary results obtained in some investigations have also been quoted. The differences may be due to a peculiar behaviour of some clay mineral and/or enzyme preparations and/or soils or to the various experimental conditions and analytical methods applied. Further research is needed to develop clay enzymology.

2. Clay minerals, through their stabilizing and protecting effect on enzymes, contribute to the accumulation of enzymes in soil, to the „survival" of enzyme molecules in the soil microenvironment. But the contribution of clay minerals to enzyme accumulation in soil and probably also in aquatic sediments is achieved in association with humic substances. The accumulated enzymes are located at microsites in or on the organo-mineral (clay-humic) complexes. Fig. 1 presents a model concerning distribution of accumulated and free enzymes in the soil microenvironment. The enzymes trapped inside the porous clay-humic complexes or attached to their surfaces are protected from proteolysis and other inactivations and yet are accessible to substrates.

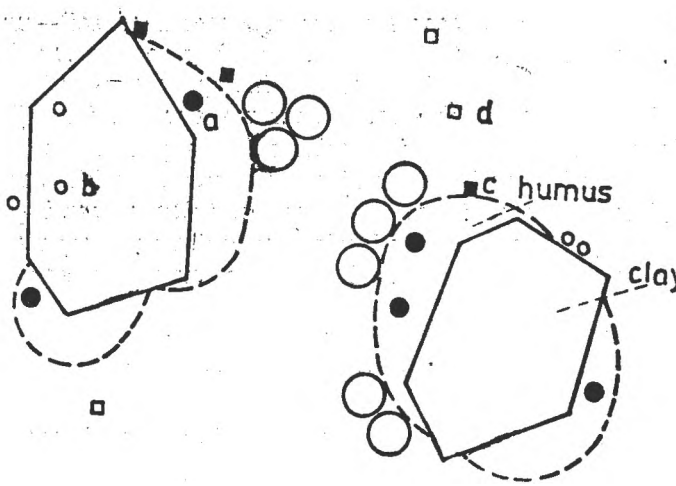

Fig. 1. *Distribution of accumulated and free enzymes in the soil microenvironment* [23, 24].
a — Enzymes trapped within or complexed with humus. *b* — Enzymes adsorbed to clay, either on the surface or between the crystal lattices. *c* — Enzymes attached to surface of organic film. *d* — Free, ephemeral enzymes in the soil aqueous phase.
The large circles represent microorganisms.

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CONTRIBUTIONS À LA CONNAISSANCE DE LA CHOROLOGIE, L'ÉCOLOGIE ET L'ÉVOLUTION DE L'ASSOCIATION *CALAMAGROSTIETUM PSEUDOPHRAGMITIS* Kopecký 1968 EN ROUMANIE

IOAN POP*, IOAN HODIŞAN* et VASILE CRISTEA*

SUMMARY. — Contributions to the Study of Distribution, Ecology and Evolution of the Association *Calamagrostietum pseudophragmitis* **Kopecký 1968 in Romania.** Reviewing literature data on the occurrence of established phytocoenoses of *Calamagrostis pseudophragmites*, the authors record the association *Calamagrostietum pseudophragmitis* Kopecký 1968 for the first time in Romania. The phytocoenoses of this association occur on the alluvial deposits of the small island lying on the river Someşul Mic close to the Cluj-Napoca Sports Ground. Ecological analyses reveal the mesohygrophilous towards hygrophilous character of these coenoses, as well as their micromesothermic and euryionic character.

Considérations générales. *Calamagrostis pseudophragmites* est une espèce de poaceae eurasiatique mésohygrophile, qui peuple — à côté d'autres plantes avec les mêmes préférences écologiques — surtout les grèves et les sables alluvionnaires des rivières, depuis la plaine et jusqu'en montagne [9].

Kopecký [4] fut le premier chercheur qui a étudié (publiant en 1968 un travail) l'écologie et la composition floristique des phytocénoses édifiées par *Calamagrostis pseudophragmites* en Tchécoslovaquie. Conformément aux observations faites sur le terrain, l'auteur considère comme écotope caractéristique pour les phytocénoses de l'association décrite les dépôts alluvionnaires (gravier, sable) du lit des rivières collinaires et de montagne, qui se trouvent sous ou au niveau annuel des eaux courantes.

Pour l'association *Rorippo-Phalaridetum arundinaceae* Kopecký 1964, Kopecký a créé en 1968 une nouvelle alliance — *Rumici-Phalaridion arundinaceae* — en indiquant comme caractéristiques les espèces suivantes: *Phalaris (Typhoides) arundinacea, Calamagrostis pseudophragmites, Rumex aquaticus, R. conglomeratus, R. obtusifolius, R. sanguineus, Barbarea stricta* et *Mentha longifolia*. Cette aliance a été integrée dans l'ordre *Nasturtio-Glycerietalia* Pign. 1953, classe des *Phragmitetea* Tx. et Prsg. 1942.

Dans notre pays, c'est à Vasiu et col. [8] qu'on attribue [6] la première mention sur les cénoses à *Calamagrostis pseudophragmites* et qui, en 1963, soulignent: „Une association qui se trouve sur des surfaces restreintes, mais qui mérite d'être mentionnée est celle de *Calamagrostis*

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pseudophragmites. Étant une plante pérennante, elle contribue à la fixation de ces sols friables . . ." (sablonneux) du Delta du Danube. À part cette citation, les auteurs ne présentent pas un relevé phytocénologique, l'espèce citée étant integrée, à côté d'autres espèces, dans des communautés arénicoles xéromésophiles présentes sur les sables maritimes.

La même année, Puşcaru-Soroceanu et col. [5] mentionnent, à leur tour, parmi les associations à poaceae d'étangs et de marais, les phytocénoses édifiées par *Calamagrostis pseudophragmites,* mais sans préciser leur composition floristique.

Beldie [1] analyse autant les communautés pures avec *Calamagrostis pseudophragmites,* que celles constituées d'un mélange hétérogène avec d'autres plantes, formées sur les graviers et les sables du lit de la vallée supérieure de Ialomiţa, qu'il intègre dans l'association *Calamagrostietum pseudophragmitis*. Le travail présente, dans 4 relevés, 15 espèces composantes de cette association, différentes au point de vue des nécessités écologiques. Dans la vallée de Ialomiţa, les communautés à *Calamagrostis pseudophragmites* ont une flore différente de celles décrites par Kopecký [3, 4, 7]. De leur composition floristique manquent les espèces caractéristiques de l'alliance, de l'ordre et de la classe dont fait partie l'association, à l'exception de la plante édifiante.

À notre avis, les communautés végétales mentionnées de la vallée de Ialomiţa représentaient à l'époque des populations de *Calamagrostis pseudophragmites* dans une phase incipiente de cénogenèse.

De même, Dihoru [2] inclut dans *Calamagrostietum pseudophragmitis* Beldie 1967, 4 phytocénoses identifiées soit sur les bords et les îlots de la rivière de Buzău, soit sur les terrains plats le long des ruisseaux de Mreaja et d'Urlătoarea. Par suite du mélange d'espèces (55 espèces) des diverses unités cénotaxonomiques, l'auteur considère comme incertaine l'appartenance de ces phytocénoses à l'association décrite par Beldie en 1967.

Sanda et col. [6] authentifient l'association *Calamagrostietum pseudophragmitis* Beldie 1967, qu'ils intègrent à tort dans l'alliance *Calthion palustris* Tx. 1937, ordre *Molinietalia* W. Koch 1926, classe *Molinio-Arrhenatheretea* Tx. 1937.

L'analyse des phytocénoses à Calamagrostis pseudophragmites **du lit de Someşul Mic, Cluj-Napoca.** Pendant le mois de juillet 1986 nous avons étudié la physionomie, l'écologie, la chorologie et la composition floristique des phytocénoses édifiées par *Calamagrostis pseudophragmites,* ayant une distribution discontinue sur les rives périodiquement inondés de Someşul Mic. Ces groupements végétaux sont localisés entre le pont du voisinage de l'hôtel Napoca, rue de Garibaldi, et le Parc sportif de l'Université. En suivrant la végétation de ce terrain, durant plusieurs années, nous avons constaté la disparition des dépôts alluvionaires et de leur végétation caractéristique, comme suite des aménagements hydrographiques effectués en 1986.

Devant le Parc sportif il y a une petite île, d'une longueur d'environ 150 m et de 8—12 m de largeur, située presqu'au milieu de la vallée

de Someșul Mic. En 1981, l'île était peuplée à la périphérie par des phytocénoses appartenant à *Calamagrostietum pseudophragmitis*, tandis que vers le centre on pouvait remarquer *Salix triandra, S. fragilis, S. alba*, coupées ultérieurement (aujourd'hui en cours de régénération). Suite de défrichage, les phytocénoses de *Calamagrostis* se sont étendues, recouvrant l'île. En même temps, le barrage qui se trouve au niveau du pont de Garibaldi favorise la crue périodique des eaux, qui submergent et déposent des alluvions sur la couche argileuse de l'île, en créant ainsi des conditions favorables pour le développement et l'extension de ces phytocénoses.

Les cénoses se présentent sous l'aspect de groupes herbacés, de 130—150 cm de hauteur avec un recouvrement de 100%. Les trois phytocénoses analysées ont dans leur composition floristique 27 espèces de cormophytes, parmi lesquelles 17 sont caractéristiques aux cénotaxons dont appartient l'association et 10 sont considérées des espèces accompagnantes (compagnes).

Les communautés végétales analysées (Tableau 1) se ressemblent beaucoup, tant sous aspect écologique que floristique, à celles de Tchécoslovaquie, ayant 10 espèces communes du total de 18 réunies dans l'association *Calamagrostietum pseudophragmitis* par Kopecký en 1968.

Tableau 1

Calamagrostietum pseudophragmitis **Kopecký 1968**

As.	Calamagrostis pseudophragmites	4	3	5
Al.	Phalaris (Typhoides) arundinacea	1	+	·
„	Rumex conglomeratus	+	+	·
„	Mentha longifolia	+	+	·
Ord.	Glyceria fluitans	+	·	+
„	Sium erectum (Berula erecta)	+	·	+
„	Epilobium parviflorum	+	·	+
Cl.	Epilobium hirsutum	+	+	·
„	Lythrum salicaria	+	·	+
„	Galium palustre	1	+	+
„	Lycopus europaeus	2	3	1
„	Mentha aquatica	+	+	+
„	M. arvensis	+	+	+
„	M. verticillata	+	·	+
„	Myosotis palustris (M. scorpioides)	+	+	+
„	Stachys palustris	+	·	+
„	Alisma plantago-aquatica	+	·	·
Com-	Polygonum hydropiper	·	+	·
pagnes	Ranunculus repens	+	+	·
„	Stellaria aquatica (Myosoton aquaticum)	+	·	+
„	Potentilla anserina	+	·	·
„	Rorippa silvestris	+	+	+
„	Calystegia sepium	+	·	·
„	Symphytum officinale ssp. uliginosum	+	·	·
„	Tussilago farfara	+	·	·
„	Poa trivialis	+	+	+
„	Equisetum arvense	+	+	·

Spectre biologique: Hh—63,0%, H—29,6%, G—7,4%.
Spectre des géoélements: Cosm—18,5%, Cp—22,2%, Eua—51,9%, E—7,4%.

Tableau 2

Le groupement écologique des espèces

Paramètre écologique*	Indices écologiques (N° et % des espèces)											
	1	1,5	2	2,5	3	3,5	4	4,5	5	5,5	6	0
U	—	—	—	—	1 3,7	1 3,7	11 40,7	4 14,8	9 33,4	—	1 3,7	—
T	—	—	—	—	19 70,4	2 7,4	1 3,7	—	—	—	—	5 18,5
R	—	—	—	—	1 3,7	—	8 29,6	—	1 3,7	—	—	17 63,0

* U—Humidité. T — Température. R — Réaction du sol.

L'espèce *Phalaris (Typhoides) arundinacea,* mentionnée par Kopecký comme une plante caractéristique à l'alliance, est aussi présente dans les phytocénoses que nous avons analysé, mais le plus souvent, elle se trouve le long de Someşul Mic en petits groupes, rappelant la cénogenèse de l'association *Rorippo-Phalaridetum arundinacae* Kopecký 1961.

Par son abondance, *Lycopus europaeus* imprime aux phytocénoses à *Calamagrostis* de l'île de Someşul Mic un *facies* caractéristique.

Le spectre écologique (Ttableau 2) relève un caracter mésohygrophile (55,5%) et hygrophile (33,4%), micro-mésotherme (77,8%) et euryionique (63,0%), en indiquant l'appartenance des phytocénoses analysées à la végétation palustre des plaines et des collines.

L'évolution des phytocénoses à *Calamagrostis pseudophragmites* dépend surtout du régime hydrique du sol.

Il en résulte donc que la vallée de Someşul Mic à Cluj-Napoca est l'écotope et la localité certaine où *Calamagrostietum pseudophragmitis* Kopecký 1968 a été identifiée jusqu'à présent dans notre pays.

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COLEOPTERE EDAFICE ŞI EPIGEE DE PE VALEA AMPOIULUI—ZLATNA ŞI MUNCEL—BAIA DE ARIEŞ

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SUMMARY. — Edaphic and Epigeic Coleoptera in the Ampoi Valley (Zlatna) and the Muncel Forest (Baia de Arieş). The paper presents 109 edaphic Coleoptera species recorded in the Ampoi valley (Zlatna) and the Muncel forest (Baia de Arieş). 57 of these species were collected from litter and soil samples, while the other 52 species were captured directly from their habitat.

40 species of the first group, represented by few specimens only, were found sporadically in the air-polluted area of the Ampoi valley, where the Zlatna industrial platform is located. 51 species, rich in specimens, were frequent in the Muncel forest, an unpolluted territory. Each of the 52 species, collected directly on soil surface in the Ampoi valley, was represented only by 1—2 specimens. These results prove that the air pollution caused by the Zlatna industrial platform greatly affects the edaphic Coleoptera.

În perioada 1979—1985, în cadrul unor cercetări privind cunoaşterea influenţei noxelor gazoase de la Zlatna asupra ecosistemelor de pe Valea Ampoiului, în aval şi amonte de această localitate, am abordat şi studiul coleopterelor edafice şi epigee în vederea urmăririi dezvoltării lor în aceste condiţii. Rezultatele din primii doi ani au scos în evidenţă o împuţinare a acestor insecte, în contextul poluării. Pentru a verifica această constatare, în perioada 1981—1985 le-am cercetat şi într-o pădure nepoluată de la Muncel-Baia de Arieş, situată la aproximativ 40 km de sursa de poluare.

Metoda de lucru. În vederea capturării acestor insecte s-a procedat la prelevarea de probe de litieră şi sol, cu rama şi sonda metalică, cît şi la culegerea lor directă.

În 1979 şi 1980, prelevările de probe au fost făcute în 7 locuri pe Valea Ampoiului: 4 în aval şi 3 în amonte de Zlatna, la distanţe de cca 5 km între ele [8]. În 1980, paralel cu prelevarea probelor, au fost culese direct din aceleaşi locuri şi coleoptere epigee. Din 1981, probele au fost luate numai din două puncte; unul notat cu 119, la 1 km aval de Zlatna, în pădure de amestec (stejar, fag şi carpen) şi altul — 142, la 15 km amonte, într-o pădure de molid cu foioase. În acelaşi an au fost luate probe de litieră şi sol dintr-un singur punct, din pădurea de făget, de la Muncel-Baia de Arieş.

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În cei 7 ani de cercetări, probele au fost prelevate de 2—5 ori pe an și de 3—6 ori pe sezon, cu excepția lunilor aprilie și octombrie, conform Tabelului 1.

Tabel 1

Prelevarea probelor pe luni și ani

Anul	Lunile						
	IV	V	VI	VII	VIII	XI	X
1979		+	+			+	
1980		+	+	+	+		+
1981			+	+	+		
1982			+	+	+	+	
1983	+		+	+		+	
1984		+	+	+			
1985		+		+			

Semnul + indică prelevările efectuate.

Pentru efectuarea analizelor am folosit lucrările [1—5, 10].

Rezultate. Au fost identificate 109 specii de coleoptere: 57 edafice (Tabel 2) și 52 epigee (Tabel 3).

Tabel 2

Coleoptere edafice

Specii	Valea Ampoiului, anii							Muncel, anii				
	79	80	81	82	83	84	85	81	82	83	84	85
Staphylinidae												
Megarthrus denticollis Beck	+							+				
Geostiba circellaris Grav.		+		+					+	+		
Quedius attenuatus Gyll.	+										+	
Othius myrmeccphilus Kiesw.							+	+	+			
Oxytelus sculpturatus Grav.							+					+
Leptusa fuliginosa Aubé										+		
„ pulchella Mannh.			+									
„ eximia Kr.				+					+	+	+	+
„ carpathica Weise			+					+		+	+	+
Medon brunneus Er.						+					+	
Acrulia inflata Gyll.			+									
Tachyporus nitidulus F.		+							+			
Aleochara moesta Grav.		+									+	
Scopaeus minutus Er.		+		+				+	+			
Austriacotiphlus piffli Sch.	+											
Stenus sylvester Er.						+				+		
„ binotatus Ljungh.											+	
Omalium rivulare Payk.						+						+
Liogluta nitidula Kr.					+			+	+			
Colydiidae												
Anommatus reitteri Gglb.				+			+			+	+	+
„ pannonicus Kaszab	+											

Tabel 2 (continuare)

Specii	Valea Ampolului, anii							Muncel, anii				
	79	80	81	82	83	84	85	81	82	83	84	85
Pselaphidae												
Biblioplectus ambiguus Reichenb.	+							+				
Trimium brevicorne Reichenb.		+		+					+	+		
Bryaxis nigripennis Aubé			+		+					+	+	+
„ reitteri Saulcy									+	+		
„ glabricollis Schm.							+				+	
„ bulbifer Reichenb.												+
Scydmaenidae												
Stenichnus godarti Latr.		+								+		
Euconnus motschulskyi Sturm								+	+			+
„ oblongus Sturm									+			
„ pubicollis Müll.								+				
Cephenium majus Reitt.										+		+
„ carpathicum Saulcy						+					+	
Lathridiidae												
Lathridius angusticollis Gyll.						+						
Cartodere filiformis Gyll.		+						+	+	+	+	
„ filum Aubé	+								+	+	+	
Corticaria pietschi Gglb.								+	+			
Corticarina gibbosa Herbst	+											+
Enicmus minutus L.						+						
Endomychidae												
Endomychus coccinaeus L.											+	
Sphaerosoma carpathicum Reitt.			+					+	+	+	+	+
„ globosum Sturm		+					+	+	+	+	+	+
„ pilosum Panz.								+				
Cryptophagidae												
Atomaria pusilla Payk.	+							+	+			+
„ fuscicollis Mannh.											+	+
Dermestidae												
Anthrenus fuscus Oliv.		+						+	+	+		
Anobiidae												
Stegobium paniceum L.	+										+	+
Curculionidae												
Phylobius argentatus L.				+						+	+	
Omias mollinus Boh.		+				+			+	+	+	+
Peritelus leucogrammus Germ.				+				+	+			+
Otiorrhynchus velutinus Germ.	+								+	+	+	
Acalles lemur Germ.										+		
„ turbatus Boh.					+		+	+	+		+	+
Tenebrionidae												
Laena reitteri Weise						+			+	+		
Nitidulidae												
Brachypterus glaber Steph.											+	
Aderidae												
Aderus populneus Panz.											+	+
Chrysomelidae												
Phytodecta quinquepunctatus F.									+			

Tabel 3

Coleoptere epigee (valea Ampoiului 1980)

Carabidae	Amara familiaris Duft.
Carabus violaceus L.	Brachinus crepitans L.
Nebria gyllenhali Schön.	*Staphilinidae*
Notiophilus biguttatus F.	Stenus biguttatus L.
Broscus cephalotes L.	„ bipunctatus Er.
Tachys sexstriatus Duft.	„ asphaltinus Er.
Bembidion lampros Herbst	„ gracilipes Er.
„ punctulatum Drap.	Paederus ruficollis F.
„ semipunctatum Don.	„ rubrothoracicus Goeze
„ tricolor F.	„ limnophilus Er.
„ tibiale Duft.	Philonthus cruentatus Gmelin
„ fasciolatum Duft.	Atheta luteipes Er.
„ decorum Zenk. Panz.	*Scarabaeidae*
„ modestum F.	Geotrupes stercorarius L.
„ transsylvanicum Bielz	Melolontha melolontha L.
„ andreae bualei Duval	*Cantharidae*
„ doderoi Gglb.	Cantharis erichsoni Bach
„ minimum F.	*Coccinellidae*
„ quadrimaculatum L.	Coccinella quinquepunctata L.
Asaphidion caraboides Schrnk.	*Chrysomelidae*
Anisodactylus signatus Panz.	Chrysomela coerulea Oliv.
Harpalus distinguendus Duft.	„ coerulans Scriba
Stenolophus teutonus Schrnk.	Labidostomis humeralis Schneid.
Acupalpus meridianus L.	Phyllodecta atrovirens Corn.
Poecilus versicolor Sturm	Haltica oleracea L.
Petrostichus melas Creutz.	*Curculionidae*
„ ovoideus Sturm	Hypera postica Gyll.
Platynus ruficornis Goeze	Rhynchaenus populi F.
Amara eurynota Panz.	Gymnetron tetrum F.
„ similata Gyll.	

Pe valea Ampoiului au fost găsite 40 specii de coleoptere edafice, prezente doar în a 10-a parte din probe și indivizi puțini. În 1979 și 1980 s-au găsit cîte 10 specii, iar în anii următori 5—6 anual.

În pădurea de la Muncel au fost găsite 51 specii, cu o frecvență anuală între 17—23 specii și un număr mai mare (normal) de indivizi.

Pe valea Ampoiului au fost culese doar 52 specii epigee, pe cînd în pădurile nepoluate ele sînt în număr mult mai mare [6, 7, 9].

Aceste fapte nu pot fi explicate decît prin influența nefavorabilă a noxelor gazoase, asupra litierei pădurilor din apropiere, locul de trai al acestor insecte și deci asupra dezvoltării lor.

Specia *Paederus rubrothoracicus* (Goeze) a fost găsită prima oară în țara noastră.

Deoarece și din punctul de vedere al coleopterofaunei edafice, valea Ampoiului cuprinde elemente deosebite, chiar rare sau foarte rare, ca *Austriacotiphlus piffli* Sch. din subfamilia *Leptotyphlinae*, semnalate pentru prima oară de noi în fauna României [8], cît și celelalte specii identificate de noi în aceste locuri, specii foarte importante în procesele edafice și în viața ecosistemelor cercetate, se impun măsuri care să ducă la reducerea sau chiar la eliminarea totală a poluării.

Concluzii. Efectele poluării din valea Ampoiului se resimt asupra speciilor de coleoptere cercetate cît și asupra numărului de indivizi. Atît speciile cît și indivizii au fost în continuă scădere în decursul anilor 1979—1985, sub influența factorilor poluanți. S-a remarcat un gradient al dinamicii numărului de specii și indivizi cercetați, dinspre zonele nepoluate spre zona poluată.

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ÜBER DIE SYSTEMATISCHE STELLUNG DER SÜD- UND WESTCHINESISCHEN UNKEN *(BOMBINA, DISCOGLOSSIDAE, AMPHIBIA)*

BOGDAN STUGREN*

SUMMARY. — On the Systematic Position of South- and West-Chinese Bombinators *(Bombina, Discoglossidae, Amphibia).* Bombinators from the provinces of Yunnan and Guangxi, South China, and Xinjiang and Szechwan, West China belong to the same species — *Bombina maxima* (Boulenger 1905), which is redescribed here on the basis of 49 specimens, preserved in various museums of the U.S.S.R., Austria, Switzerland, Federal Republic of Germany, U.K., and the U.S.A. The name *B. microdeladigitora* Liu, Hu and Yang 1960 does not designate an independent taxon, being only a colour pattern variation without nomenclatural validity inside the species *B. maxima*.

Aus Südchina wurden zwei Arten der euro-ostasiatischen Discoglossidengattung *Bombina* beschrieben: *B. maxima* (Boulenger 1905) und *B. microdeladigitora* Liu, Hu und Yang 1960. Das Ausbreitungsgebiet von *B. maxima* erstreckt sich auf Hochgebirgen in Yunnan, SW-Sichuan und SO-Xinjiang, ferner auf den westlichen Teil von Guizhou; ausserhalb Chinas umfasst es den nördlichen Teil der Provinz Bac-bo in Vietnam [4]. In dem mir zugänglichen Material gibt es auch ein Stück aus Wutang, Provinz Guangxi. Dadurch wird gezeigt, dass das Areal der Art sich weiter südöstlich als die südchinesischen Hochgebirgszüge erstreckt, ohne jedoch die Küste des Südchinesischen Meeres zu erreichen. Dagegen ist *B. microdeladigitora* nur aus der Terra typica (Huang-tsiao-ling, Chung-tung, Yunnan) bekannt, soll also inmitten des Areals von *B. maxima* eingebettet sein. Hier wird die Frage aufgeworfen ob es tatsächlich in Süd- und Westchina zwei *Bombina*-Arten gibt.

Bei europäischen Unken (*B. variegata* (L.) und *B. bombina* (L.)) sind Zeichnungsvariationen (verschiedene Flächenverhältnisse der schwarzen und hellen Farben auf der Körperunterseite) als genetisch bedingter Polymorphismus aufgefasst [8, 10]. Darüber sind jedoch Literaturangaben für *B. maxima* sehr spärlich. Boulengers [2] Originalbeschreibung stützt sich auf drei Stücke und besagt nur, dass auf der Körperunterseite entweder Orange und Schwarz gleiche Flächenanteile einnehmen oder schwarzes Feld vorherrschend ist**. Liu [4], der 29 Stücke untersuchte, gibt auch kein statistisches Bild der Variationen der Ventralzeichnung. Die Art *B. microdeladigitora* soll sich von *B. maxima* durch vorwiegend schwarzgefärbte Körperunterseite unterscheiden*** [5]. Hier

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** Der englische Originaltext lautet: „Lower parts marbled bright orange in about equal proportions, or the black predominating."

*** Im Originaltext: „B ack color dominant on the ventral side of the body".

wird versucht, auf Grund von 49 Stücke, die Variationen der Zeichnungen auf der Körperunterseite statistisch zu beurteilen. Die Farben wurden hier nur als schwarz und bzw. hell angeführt, da bei dem alten, konservierten Material die Originalfarben verwischt sind. Darüber hinaus wird auch die Variation der Körperproportionen statistisch dargelegt.

Material. Insgesamt 49 Stücke aus folgenden Sammlungen*:

Lehrstuhl für Zoologie der Wirbeltiere an der Universität Leningrad (UL) 1 Stück: (keine Katalognummer, keine Sammlerangabe), Yunnan;

Naturhistorisches Museum Wien (NHMW) 8 Stücke: Nr. 6 812 Südchina (ohne nähere Fundortangabe), leg. F. Werner, 1933; Nr. 6 813/1—4 Yunnan Fu**, leg. F. Steindachner, 1909; Nr. 6 814: 3 Tongekuan Fu, Westchina, leg. F. Steindachner, 14.2. 1906; Nr. 6 815/1—2 Lidjiang, NW-Yunnan, leg. Handel-Mazzetti, 6. 1915;

Naturhistorisches Museum Basel (NHMB) 1 Stück: Nr. 306 Tongchuan Fu, Yunnan, ein Topotyp (keine Sammlerangabe);

Senckenberg-Museum, Frankfurt/Main (SMF) 2 Stücke: Nr. 1 439 Yunnan, leg. Zoologisches Museum Berlin, 1925; Nr. 29 672 Tongchuan, Fu, Yunnan, leg. F. Werner, 1933;

Zoologisches Museum Hamburg (ZMH) 2 Stücke: Nr. 1 659 und 6 294 Yunnan, leg. F. Werner, 1911;

British Museum (Natural History), London (BMNH) 21 Stücke: Nr. 52 913—52 926 Tongchuan Fu, Yunnan (keine Sammlerangabe), 1906; Nr. 52 927—52 928 ibid. (keine Sammlerangabe), 1926; Nr. 52 929 ibid. (keine Sammlerangabe), 1909;

United States National Museum, Washington, D.C. (USNM) 2 Stücke: Nr. 124 578—124 579 Sichang, Sikiang, W-China, leg. C. C. Liu, 5. 1942.

American Museum of Natural History, New York (AMNH) 4 Stücke: Nr. 5 445 Yunnan Fu, Yunnan, leg. J. Graham, 2. 1919; Nr. 8 148 Wutang Chow District, Kwangsi, leg. J. Graham, 26. 7. 1920;

Museum of Comparative Zoology at Harvard College, Cambridge, Mass. (MCZH) 4 Stücke: Nr. 2 466/1—2 Tonchuan Fu, Yunnan (keine Sammlerangabe); Nr. 9 618—9 619 Yunnan Fu, Yunnan (keine Sammlerangabe);

Field Museum of Natural History, Chicago, Ill. (FMNH) 5 Stücke: Nr. 7 935—7 936 Yunnan Fu, Yunnan, leg. J. Graham (keine Zeitangabe); Nr. 49 519—49 520 und 49 522 Sikiang, W-China, leg. C. C. Liu, 5. 1942.

Ergebnisse. Das untersuchte Material zeigt verschiedenartige schwarz-hell-Flächenverhältnisse auf der Körperunterseite, die in 8 Formen eingeteilt werden können, welche ich von A bis H bezeichne (Abb. 1). Bei einigen Stücken (AMNH Nr. 5 445; BMNH Nr. 52 923) ist die Unterseite des Körpers von einem grossen, hellen Feld eingenommen, das sich von den Zehenspitzen der Hinterfüsse bis zur Spitze des Unterkiefers ausdehnt. Auf diesem Feld sind hier und da schwarze Flecken verstreut. Diese Form bezeichne ich als A. Das entgegengesetzte Extrem stellt die H-Form dar, bei welcher ein schwarzes Feld die Unterseite des Körpers

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** Um Verwechslungen zu vermeiden, wird hier die heute nicht mehr übliche Schreibweise chinesischer Ortsnamen genau nach den Etiketten der Museumsstücke wiedergegeben. Sonst im Text werden chinesische Provinznamen nach der gegenwärtigen Transkription geschrieben, z. B. Xinjiang statt Sinkiang.

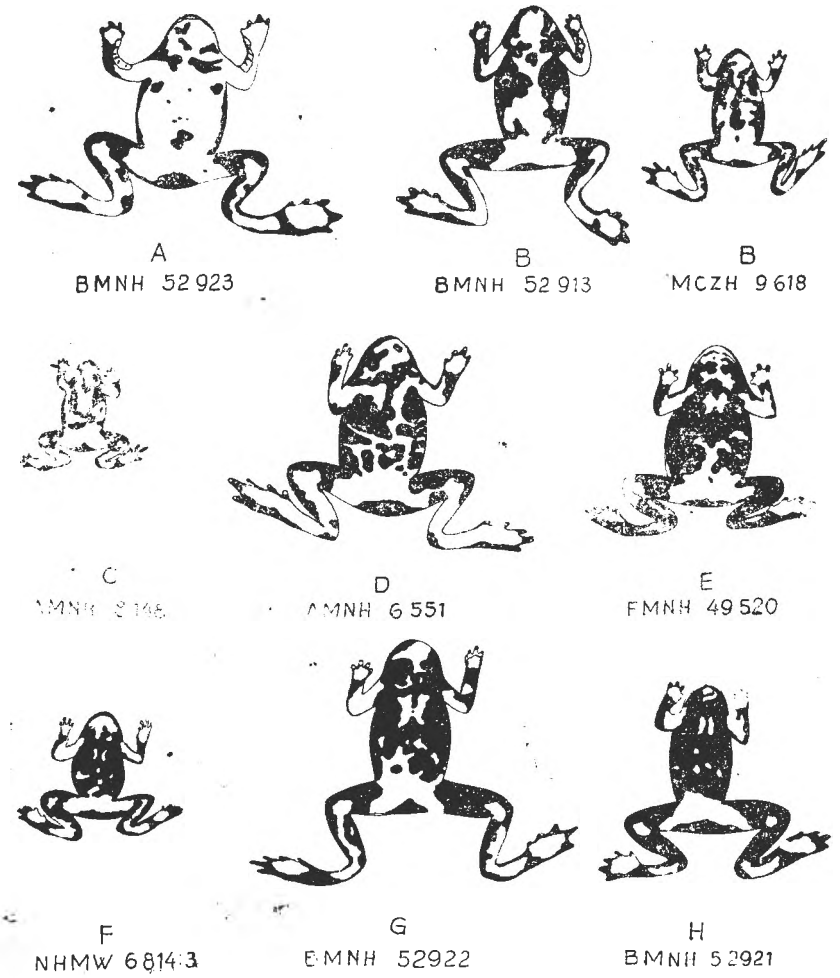

Abb. 1. *Zeichnungsvariationen der Körperunterseite bei* Bombina maxima. *A — H* — Verschiedenartige Formen des Verhältnisses von hellen und schwarzen Feldern auf der Körperunterseite. Abkürzungen s. im Text. Nummern — Katalognummern in den untersuchten Museums-sammlungen.

einnimmt und bloss auf der Innenseite der Hüften ein grösserer heller Fleck vorkommt. Übrigens ist das schwarze Feld mit kleinen hellen Flecken und hellen Punkten durchsät. Zwischen den extremen Formen A und H kommen alle möglichen Übergangsformen vor. Von der Form A ausgehend, wird das helle Ventralfeld allmählich reduziert. Die Form B ist noch durch das Überwiegen der hellen Fläche charakterisiert (z. B. BMNH Nr. 52 915 und 52 925; MCZH Nr. 9 618). Bei der Form C (AMNH Nr. 8 14ð) nimmt das helle und das schwarze Feld fast die gleiche

Fläche ein. Bei der Form D (AMNH Nr. 6 551) geht die gelbe Fläche vielmehr zurück. Bei der Form E (FMNH Nr. 49 520: ZMH Nr. 1659; NHMW Nr. 6 812) ist das helle Feld schon stark reduziert. Bei der Form F (NHMW Nr. 6 814: 3) ist das helle Feld der Ventralseite auf mehrere kleine Flecke gespaltet, wobei auf der Brust zwei klare parallel zueinander stehende helle linsenförmige Flecken vorhanden sind. Bei der Form G (BMNH Nr. 52 922) ist die Ventralseite des Körpers schwarz mit hellen Punkten. Wie oben schon gesagt, werden bei der Form H (BMNH Nr. 52 921) die hellen Flecken noch stärker reduziert.

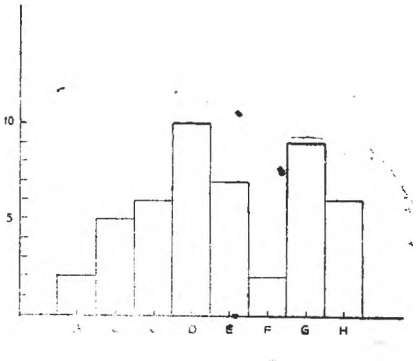

Abb. 2. *Häufigkeitsverteilungen der Zeichnungsvariationen der Körperunterseite bei* Bombina maxima.
Abszisse — Verschiedenartige Formen des Verhältnisses von hellen und schwarzen Feldern auf der Körperunterseite. Ordinate — Entsprechende Häufigkeiten.

Aus Abb. 2 geht hervor, dass die meisten Stücke jenen Formen angehören, bei welchen das schwarze und helle Feld im fast gleichen Verhältnis zueinander stehen. Die extreme Form A kommt selten vor. Die extreme Form H ist aber häufiger vorhanden. Bei allen untersuchten Stücken fliessen die hellen Hüftflecken von der rechten und linken Seite in ein einheitliches Feld zusammen. Bei 22 Stücken ist der Tarsalfleck vom Plantarfleck an beiden Extremitäten getrennt, bei 12 Stücken dagegen beiderseits zusammenfliessend, während bei 13 Stücken auf einer Seite Tarsal- und Plantarfleck getrennt sind, auf der anderen Seite dagegen zusammenfliessen. Bei weiteren Stücken (NHMW Nr. 6 815: 1—2) war die Zeichnung der Ventralseite unbeurteilbar, da die Konservierungsflüssigkeit Farbe und Zeichnung gänzlich abgewischt hat.

Was die Körperproportionen anbelangt (Tabelle 1), so unterliegt die Kopf+Rump—Länge (KRL) weitgehenden Schwankungen von 30 bis 70 mm (Mittelwert 55,20), während das Femur/Tibia-Verhältnis (F./T.) 0,84—1,39 beträgt.

Diskussion. Wie oben schon erwähnt, weisen süd- und westchinesische Unken hohen Polymorphismus hinsichtlich der Zeichnungsvariationen auf. Auf Grund der schwarz-hell-Verhältnisse der Körperunterseite könnte man leicht mehrere „Arten" beschreiben. Da jedoch verschiedene Zeichnungsvariationen aus derselben Stichprobe stammen und offenbar dieselben natürlichen Populationen vertreten, möchte ich ein solches Verfahren nicht vorschlagen. Deswegen kann ich mich nicht der Meinung von Liu, Hu und Yang [5] anschliessen, wonach es in Yunnan ausser *B. maxima* eine weitere Unkenart, *B. microdeladigitora*

Tabelle 1

Körperproportionen und Zeichnungsvariationen der Ventralseite bei *Bombina maxima*

KRL — Kopf + Rumpf-Länge. *F.* — Femur-Länge. *T.* — Tibia-Länge (alle Masse in mm).
Hf — Heller Hüftfleck. *Bf* — Heller Brustfleck. *Tf* — Heller Tarsalfleck. *Pf* — Heller Plantarfleck. *v* — Hf zusammenfliessend, Bf ungeteilt, Tf mit Pf vereint. *b* — Hf geteilt, Bf in zwei linsenförmige Teile gespaltet, Tf und Pf voneinander getrennt. *r* — Rechts; *l* — Links. *A* bis *H* — Formen der Zeichnungsvariationen (nähere Erklärungen s. im Text).

Katalognummer	No.	KRL	F.	T.	Hf	Bf	Tf/Pf		Form
							r	l	
1	2	3	4	5	6	7	8	9	10
UL	1	—	—	—	v	b	b	b	D
NHMW 6 812	2	55	28	25	v	b	b	v	D
— 6 813/1	3	66	29	26	v	v	b	b	G
— 6 813/2	4	50	24	21	v	v	b	v	C
— 6 813/3	5	43	21	19	v	v	b	b	G
— 6 813/4	6	52	23	19	v	v	v	b	G
— 6 814 : 3	7	65	31	26	v	b	b	b	H
— 6 815 : 1	8	62	31	27	Zeichnung nicht erkennbar				
— 6 815 : 2	9	58	29	24	"	"	"		
SMF 1 439	10 ♂	54	24	23	v	v	b	b	E
—29 672	11 ♂	55	23	24	v	b	v	b	D
ZMH 1 659	12	50	20	20	v	v	v	v	E
NHMB 306	13	55	19	21	v	v	b	v	C
BMNH 52 913	14 ♂	46	18	18	v	b	b	b	H
—52 914	15 ♂	70	33	29	v	b	b	b	G
—52 915	16 ♂	59	28	26	v	v	v	v	B
BMNH 52 916	17	59	23	23	v	b	v	v	G
—52 917	18	30	12	12	v	v	v	v	C
—52 918	19 ♂	61	29	29	v	v	b	b	D
—52 919	20 ♂	55	27	25	v	v	b	b	C
—52 920	21 ♂	43	18	21	v	v	b	b	G
—52 921	22	52	25	24	v	b	b	b	H
—52 922	23	62	22	23	v	v	b	b	G
—52 923	24 ♂	68	34	32	v	v	b	b	A
— 52 924	25	55	21	19	v	v	v	v	D
— 52 925	26	61	27	23	v	v	v	v	B
— 52 926	27	59	24	24	v	b	v	v	D
— 52 927	28	31	11	13	v	v	v	v	C
— 52 928	29	28	11	12	v	v	v	b	E
— 52 929	30	25	10	10	v	v	v	v	D
—103 016	31 ♂	62	27	29	v	v	b	b	E
—103 017	32 ♂	60	28	29	v	v	v	b	E
—103 018	33 ♂	59	29	27	v	b	b	b	H
—103 019	34 ♂	52	24	23	v	v	v	v	D
USNM 124 578	35 ♂	62	28	26	v	b	b	b	E
—124 579	36	61	26	27	v	v	b	b	F
AMNH 5 445	37	37	16	15	v	v	v	v	A
— 6 550	38 ♂	48	21	20	v	b	b	b	H
— 6 551	39	57	25	20	v	v	b	b	D
— 8 148	40	26	10	10	v	v	v	b	C

Tabelle 1 (Fortsetzung)

1	2	3	4	5	6	7	8	9	10
MCZH 2 466/1	41 ♂	52	21	19	v	v	b	b	G
— 2 466/2	42 ♂	50	22	22	v	v	v	v	H
— 9 618	43	55	23	21	v	v	v	v	B
— 9 619	44	55	24	20	v	b	b	b	G
FMNH 7 935	45	60	21	22	v	b	b	v	B
— 7 936	46	41	19	18	v	b	b	b	B
— 49 519	47 ♂	68	30	23	v	v	b	v	D
— 49 520	48	68	29	21	v	v	b	v	D
— 49 522	49	60	21	22	v	v	b	v	E

gäbe. Die letztere „Art" ist meines Erachtens kaum als valider Taxon zu betrachten, sondern nur als Zeichnungsvariation der Unterseite von *B. maxima* aufzufassen. Daraus folgt, dass der Name *B. microdeladigitora* zur Synonymenliste von *B. maxima* gehört. Da ferner keine morphologische Unterschiede zwischen Unken aus West- und Südchina festgestellt werden konnten, so wird hier die Vielfalt der Zeichnungsvariationen bei *B. maxima* als Ausdruck des genetischen Polymorphismus aufgefasst.

Die Aufspaltung der Populationen in ortsunabhängige Zeichnungsvariationen ist übrigens bei europäischen Unken (Abb. 3) und auch bei der Fernöstlichen Unke *B. orientalis* (B o u l e n g e r) (Abb. 4) in Koreea und dem Ussuri-Land (UdSSR) eine allgemeine Erscheinung [7, 8].

A b b. 3. *Zeichnungsvariationen der Körperunterseite bei* Bombina variegata *aus Südosteuropa* [10].
A — H — Verschiedenartige Formen des Verhältnisses von hellen und schwarzen Feldern auf der Körperunterseite.

Abb. 4. *Die häufigste Zeichnungsvariation der Körperunterseite bei der Fernöstlichen Unke* (Bombina orientalis) [1].

Ob die hier aufgestellten morphologische Reihe der Zeichnungsvariationen von heller bis fast ganz schwarzer Ventralseite auch eine Evolutionsreihe darstellt, lässt sich anhand der hier veröffentlichten Tatsachen nicht entscheiden. Im Vergleich zu den Zeichnungsvariationen der europäischen Unken, lässt sich bloss eine Vermutung über die Evolution der Gattung *Bombina* in Ostasien aufstellen. Laut Mertens [6] soll *B. maxima* ein Gegenstück zu der südosteuropäischen Gelbbauchunke *(B. variegata)* sein. Bei beiden Arten sind die hellen Hüftflecke nicht gespaltet und die Variation der Bauchzeichnungen sehr ähnlich. Die Gelbbauchunke weist einen unverkennbaren Trend zur Reduktion der hellen Fläche auf der Körperunterseite von Griechenland aus in Richtung Nord und Nordwest [9]. Stücke mit ausgedehntem hellem Feld auf der Körperunterseite sind aber, wie schon erwähnt, bei *B. maxima* äusserst selten. Stücke mit ausgedehntem schwarzem Feld kommen aber häufig vor. Von einem evolutiven Trend zur Reduktion der hellen Fläche auf der Körperunterseite kann man bei *B. maxima* nicht reden. *B. maxima* weist eher Merkmale, welche auch an *B. bombina* erinnern: der oft vorkommende, in zwei linsenförmige Teile gespaltete helle Brustfleck, sowie die häufige Trennung von Tarsal- und Plantarfleck.

Obwohl Boulenger [2] sehr wenig Material aus Yunnan untersuchte, hat seine Originalbeschreibung von *B. maxima* das Wesentliche der Zeichnungsvariationen bei dieser Art hervorgehoben: die Ventralseite ist entweder im gleichen Verhältnis schwarz und hell gefärbt, oder nimmt das schwarze Feld eine grössere Fläche ein. Die hier aufgeführten Beschreibungen eines reichen Materials aus mehreren Provinzen Süd- und Westchinas gestatten nur dieselbe Schlussfolgerung.

Ob *B. maxima* der unbekannten voreiszeitlichen Stammform der Gattung *Bombina* näher als andere *Bombina*-Arten steht, ist schwer zu entscheiden. Der Gedanke ist verlockend, ruft jedoch unüberprüfbare Spekulationen hervor. Inwieweit die Aufspaltung der Stammform in mehrere Arten durch die Glazialzeiten bedingt wurde, lässt sich aus Mangel von Fossilien nicht leicht beurteilen.

Durch die Vergletscherung Sibiriens im Quartär wurde wahrscheinlich das Verbreitungsgebiet der Gattung *Bombina* in einen europäischen und einen ostasiatischen Teil gespaltet. Die Evolution der Unken in China und dem Amurgebiet war wohl mit der ostasiatischen Wald-

geschichte verbunden. Das ostasiatische Waldgebiet wurde von der Eiszeit beeinträchtigt und beeinflusst [3]. Die südchinesischen Gebirgszüge trugen Vorlandvergletcherungen im Pleistozän, was zur Versteppung der Landschaft und zur Entstehung bedeutender Lösslager während der letzten Kaltzeit im Mittleren China führte [11]. Dadurch erfolgte ein Rückgang des Waldes im Unterlauf des Yang-tse. Somit entstanden zwei Teilgebiete des ostasiatischen Areals der Unken, im Amurbecken und in Südchina. Dadurch wurde die geographische Vorbedingung der weiteren Artbildung geschaffen. In jedem isolierten Teilgebiet entstand eine besondere Art: *B. orientalis* im Norden, *B. maxima* im Süden.

Schlussfolgerungen. Auf Grund der Untersuchung von 49 Stücken aus Süd- und Westchina, werden süd- und westchinesische Unken als eine einzige Art, *B. maxima* (Boulenger) betrachtet. Der Name *B. microdeladigitora* Liu, Hu und Yang wird nicht anerkannt und in die Synonymenliste von *B. maxima* übergeführt. Die Zeichnungsvariationen der Körperunterseite werden als Ausdruck des genetischen Polymorphismus aufgefasst. Zum erstenmal wird die Spezies *B. maxima* auch anhand von Körperproportionen charakterisiert.

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THE INFLUENCE OF COMPLEX TREATMENT WITH GAMMA RADIATIONS, COPPER AND CADMIUM ON THE RNA CONTENT IN MAIZE SEEDLINGS

LENUȚA RÁKOSY-TICAN* and MIHAI TRIFU**

SUMMARY. — The influence of γ-radiations in the presence of Cu and Cd on the RNA content of maize radicles was studied. The following irradiation doses were used: 200, 400, 1,200, 2,000 and 4,000 rad. The microelements were administered as 0.005 and 0.0005% $CuSO_4$ solutions and 0.0005 and 0.00005% $Cd(NO_3)_2$ solutions, respectively. All possible combinations among these three factors were tested. It has been found that Cu and Cd, especially in the combination 0.005% $CuSO_4$ plus 0.0005% ($Cd(NO_3)_2$, may be used for diminishing the effect of high doses of γ-radiations to reduce the RNA content in organisms.

The study of the influence of low doses of ionizing radiations on plants is motivated, on the one hand, by the interest in establishing optimum biostimulating doses [4] and, on the other hand, by the necessity of knowing their mechanisms of action in relation to the continually ascending level of radioactivity on Earth as a result of atomic energy utilization in various fields of human activity.

The complex influence of γ-radiations and microelements on plants [1, 2, 7, 8] appears to be of special interest, since in nature physical and chemical agents do not act separately but interact with each other, revealing various aspects of synergism or antagonism. Moreover, some microelements display a protective effect against the noxious influence of radiations on plants.

In a previous paper [7] we initiated the study of the influence of γ-radiations emitted by ^{60}Co, between 200 and 4,000 rad, as well as that of 0.005% and 0.0005% $CuSO_4$ solutions and 0.0005% and 0.00005% $Cd(NO_3)_2$ solutions on the RNA content in the radicles of maize seedlings. Using the same concentrations of the two microelements and the same irradiation doses, in the present study we have focussed our attention on the complex influence of all three factors, in all possible combinations, on the RNA content in the radicles of maize seedlings.

Materials and method. The researches have been carried out with the early hybrid of HD-101 maize, whose dry caryopses were subjected to γ-irradiation emitted by ^{60}Co for 30 seconds (200 rad), one minute (400 rad), 3 minutes (1,200 rad), 5 minutes (2,000 rad) and 10 minutes (4,000 rad). After irradiation the caryopses were imbibed with $CuSO_4$ and $Cd(NO_3)_2$ solutions or with distilled water for 24 hours and then put to germinate in germinators (100 caryopses/germinator) on filter paper moistened with the 4 solutions or with distilled water.

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The following variants were experimented:
— Control (unirradiated and untreated with microelements);
— A : γ-radiations only (no microelements);
— B : ±γ-radiations + 0.005% $CuSO_4$ + 0.00005% $Cd(NO_3)_2$;
— C : ±γ-radiations + 0.005% $CuSO_4$ + 0.0005% $Cd(NO_3)_2$;
— D : ±γ-radiations + 0.0005% $CuSO_4$ + 0.00005% $Cd(NO_3)_2$;
— E : ±γ-radiations + 0.0005% $CuSO_4$ + 0.0005% $Cd(NO_3)_2$.

Germination and growth of seedlings took place at room temperature (22±3°C), the filter paper being moistened daily. The RNA content of 3, 6 and 9 days old seedlings was assayed by the Cherry method [3]. The determinations were performed with a VSU-1 spectrophotometer, while the RNA absorption curves were recorded by means of a Specord UV—VIS spectrophotometer.

The data were statistically processed by using the multiple *t* test [5].

Results and discussion. It has been noticed that small doses of γ-radiations bring about a slight increase in the RNA content of radicles in maize seedlings (Table 1), which is statistically significant for the

Table 1

Influence of gamma radiations, Cu and Cd treatment on RNA content in maize seedlings

Variant	Irradiation dose (rad)	RNA (mg/g dry substance)		
		Plant age (days)		
		3	6	9
Control	0	38.2±0.83	36.4±0.32	35.8±0.40
A	200	39.6±0.34	39.2±0.54	36.7±0.68
	400	39.0±0.85	37.1±0.36	36.4±0.43
	1,200	31.6±0.44	31.7±0.32	28.6±0.83
	2,000	30.2±0.39	29.6±0.44	27.3±0.64
	4,000	25.3±0.63	25.1±0.32	24.6±0.86
B	0	34.6±0.73	34.0±0.33	31.8±0.92
	200	40.3±0.39	38.0±0.53	36.2±0.24
	400	39.6±0.80	38.1±0.29	34.2±0.32
	1,200	36.3±0.42	36.1±0.32	31.3±0.42
	2,000	32.6±0.74	31.3±0.88	27.2±0.38
	4,000	29.3±0.42	31.3±0.24	25.6±0.32
C	0	35.4±0.88	35.6±0.32	32.6±0.75
	200	39.2±0.46	37.6±0.26	34.6±0.92
	400	39.2±0.49	37.8±0.62	32.1±0.75
	1,200	37.6±0.54	36.8±0.42	33.7±0.26
	2,000	34.1±0.55	33.0±0.26	26.1±0.83
	4,000	30.6±0.36	29.4±0.48	25.8±0.47
D	0	37.6±0.42	36.1±0.25	31.4±0.42
	200	39.2±0.35	39.4±0.36	35.4±0.23
	400	38.6±0.84	37.2±0.88	33.6±0.32
	1,200	36.5±0.27	35.6±0.26	30.3±0.64
	2,000	34.3±0.95	33.1±0.83	29.2±0.32
	4,000	29.6±0.28	27.2±0.87	25.3±0.42
E	0	38.0±0.62	37.0±0.42	32.2±0.96
	200	39.4±0.42	38.4±0.26	36.1±0.42
	400	38.2±0.43	37.2±0.42	33.7±0.42
	1,200	37.2 ±0.40	34.7±0.45	31.2±0.43
	2,000	33.6±0.48	31.9±0.67	28.8±0.23
	4,000	28,3±0.32	25.9±0.70	26.1±0.33

200 rad dose in 3 and 6 days old seedlings. This significance decreases or even disappears in 9 days old seedlings. For the 400 rad dose the differences are not significant (Table 2). Doses larger than 1,200 rad induce a decrease in the RNA content of radicles (Table 1), which is statistically extremely significant for all the three ages studied (Table 2).

Table 2

Statistical significance of the differences between mean values
(Multiple *t* test [5]).

Plant age (days)	Irradiation dose (rad)	Significance of the differences between mean values as compared to control					Significance of the differences between mean values as compared to variant A			
		A	B	C	D	E	B	C	D	E
3	0	—	vs	s	n	n	vs	vs	n	n
	200	s	vs	s	s	s	l	n	n	n
	400	n	s	n	n	n	n	n	n	n
	1,200	vs	vs	n	s	s	vs	vs	vs	vs
	2,000	vs	vs	vs	vs	vs	s	vs	vs	vs
	4,000	vs	vs	vs	vs	vs	vs	vs	vs	vs
6	0	—	vs	s	n	s	vs	s	n	s
	200	vs	vs	s	vs	vs	s	vs	n	s
	400	n	s	s	l	l	s	n	n	n
	1,200	vs	n	n	s	vs	vs	vs	vs	vs
	2,000	vs	vs	vs	vs	vs	s	vs	vs	vs
	4,000	vs	vs	vs	vs	vs	vs	vs	vs	vs
9	0	—	vs	vs	vs	vs	vs	vs	vs	vs
	200	l	n	s	n	n	n	s	s	n
	400	n	vs	vs	vs	vs	vs	vs	vs	vs
	1,200	vs	vs	vs	vs	vs	vs	vs	v	vs
	2,000	vs	vs	vs	vs	vs	n	s	s	s
	4,000	vs	vs	vs	vs	vs	s	s	n	s

vs – Very significant. *s* — Significant. *n* — Non-significant. *l* — Limit.

The 4 combinations of microelements (B, C, D and E) have a slightly inhibitory influence on the RNA content of radicles in the absence of irradiation (Table 1). This influence becomes very significant with the aging of seedlings (Table 2).

When compared to mere irradiation treatments, complex treatments reveal a tendency to diminish the inhibitory effect of large irradiation doses. This effect decreases with the age, so that very significant differences become significant or even non-significant in 9 days old seedlings for D treatment, as compared to mere irradiation treatments (Table 2). This suggests that treatments with microelements may protect seedlings against tho noxious influence of γ-radiations.

Regardless of treatments, the RNA content of radicles generally decreases with the growth of seedlings [9].

Conclusions. Although the mechanism of interaction among these factors at intracellular level is less known for the time being, the applied

combinations of microelements seem to exert a protective effect against the noxious influence of γ-radiations, influencing perhaps some enzymatic processes [6]. Future researches will establish whether this protective effect holds good for other microelements as well and to what extent the effect persists during the whole period of vegetation.

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STUDIA UNIV. BABEŞ—BOLYAI, BIOLOGIA, XXXI, 2, 1986

FREE AMINO ACIDS IN FLOWERS OF SOME VERNAL PLANTS

TIBERIU PERSECĂ* and MARCEL PÂRVU**

SUMMARY. — The free amino acids were extracted from flowers of *Cornus mas* L., *Anemone nemorosa* L., *Corydalis solida* L., *Bellis perennis* L., *Tussilago farfara* L. and *Primula acaulis* L. by homogenization and decoction, then analyzed by one- and two-dimensional paper chromotography. Large amounts of γ-aminobutyric acid, alanine, serine and glycine were found in flowers of all studied species. The *Cornus mas* and *Anemone nemorosa* flowers contained the largest and the flowers of *Tussilago farfara* and *Primula acaulis* the smallest amounts of free amino acids. On the chromatogram of the *Corydalis solida* flower extract, a spot appeared near that of proline. It seems that the unidentified ninhydrin-positive compound producing this spot is characteristic for *Corydalis solida*.

The pattern of proteic amino acids present in acid hydrolyzates of homogenates and decoctions showed small differences from one species to another.

Under the climatic conditions of our country, the source of pollen and nectar for bees in the March—April period is provided by a relatively small number of plants. Among these we mention 6 species: *Cornus mas* L., *Anemone nemorosa* L., *Corydalis solida* L., *Bellis perennis* L., *Tussilago farfara* L. and *Primula acaulis* L. These species are valuable medicinal plants.

As a continuation of our previous studies [8—11], we determined the content of free amino acids (FAA) and of proteic amino acids (PAA) in the flowers of these 6 plants.

Material and methods. In April, flowers were collected from plants growing in the Criş valley, then dried to constant weight. The FAA were extracted by homogenization of the dried flowers and by decoction. The PAA were obtained following acid hydrolysis of the proteic precipitates. Both FAA and PAA were analyzed by one- and two-dimensional paper chromatography. The methods of extraction and chromatography were already described in detail [11].

In order to compare the results with those obtained with other species, the same amounts of extracts were applied on chromatographic papers as previously [8—11].

Results and discussion. All FAA present in the extracts obtained by homogenization of dried flowers were also found in decoctions but in smaller amounts. The decoctions contained solved proteins, too. The number of spots appearing on two-dimensional chromatograms (Fig. 1) varied with different plants as follows: *Cornus mas* — 22, *Anemone nemorosa, Corydalis solida* and *Primula acaulis* — 18, *Bellis perennis*

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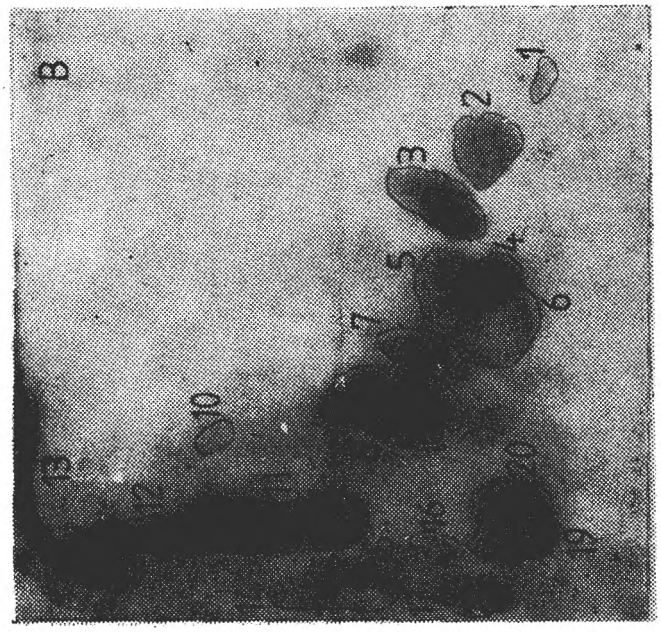
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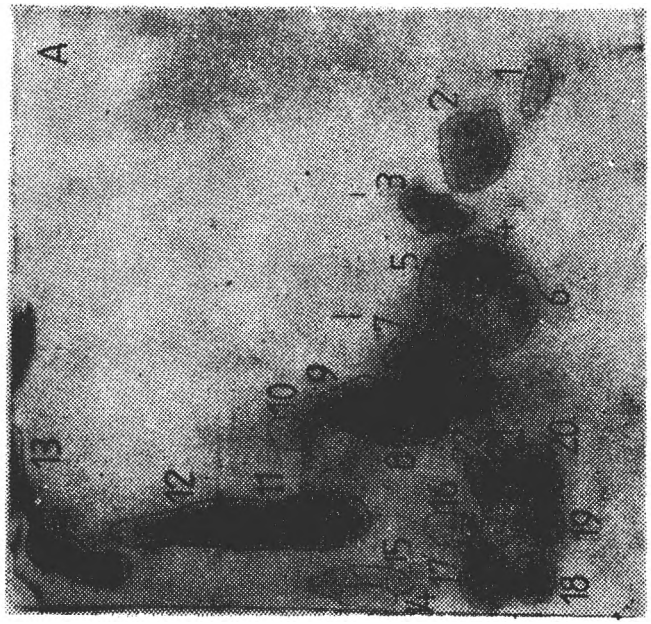
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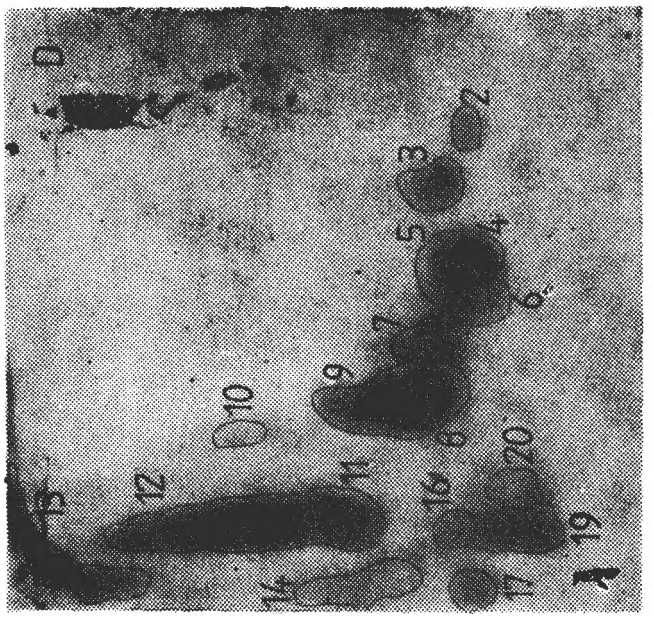
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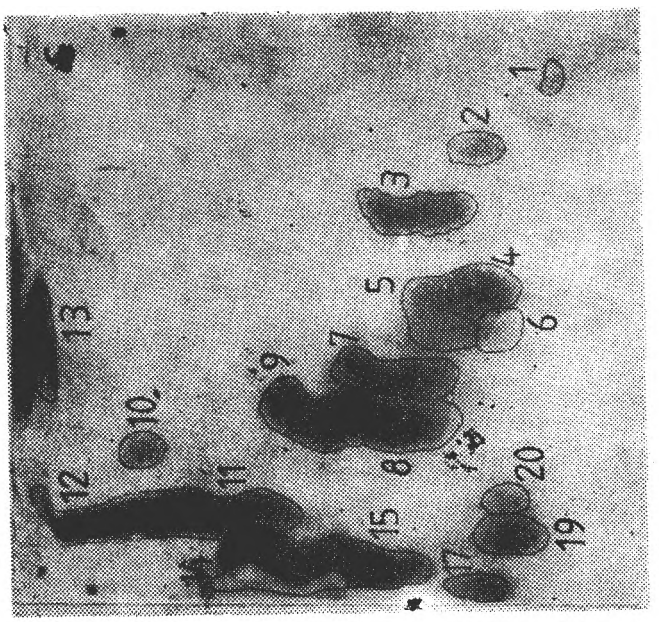
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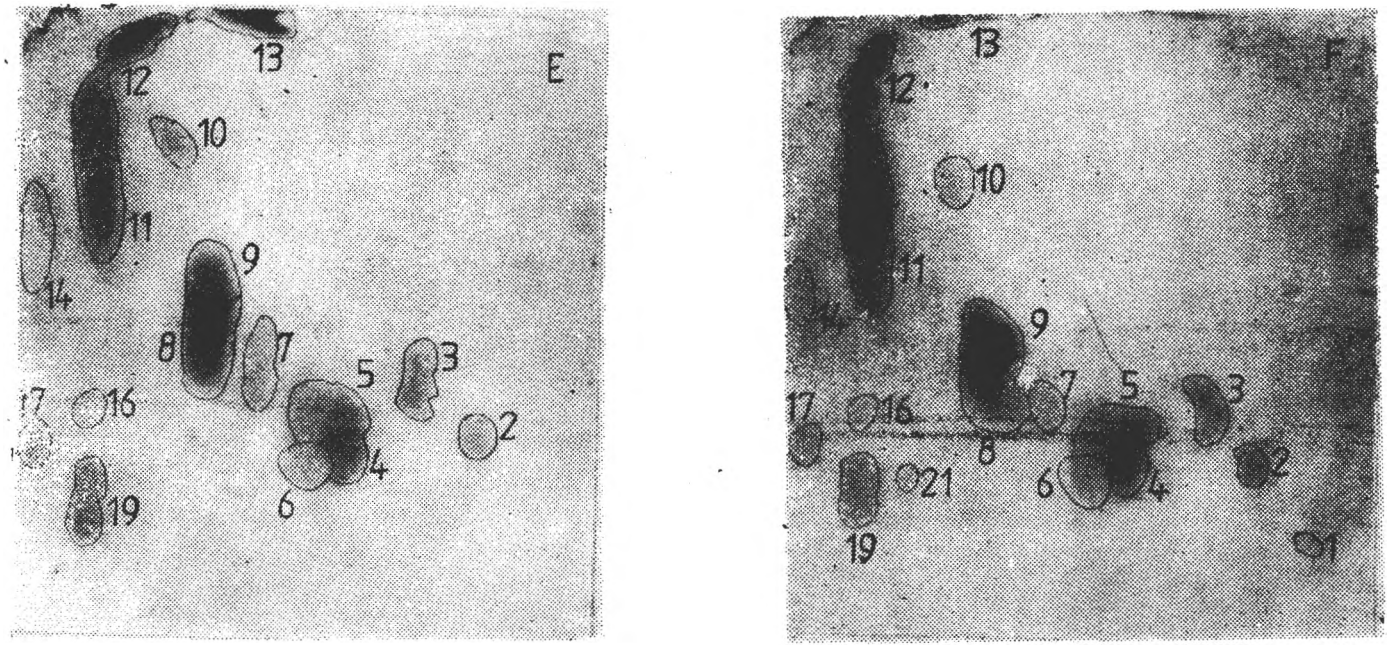

Fig. 1. *FAA in flowers of some vernal plants.*
A — *Cornus mas* L. *B* — *Anemone nemorosa* L. *C* — *Corydalis solida* L. *D* — *Bellis perennis* L. *E* — *Tussilago farfara* L. *F* — *Primula acaulis* L.
1 — Cysteic acid. *2* — Aspartic acid. 3 — Glutamic acid. *4* — Serine. *5* — Glycine. *6* — Asparagine. *7* — Threonine. *8* — Alanine. *9* — β-Alanine. *10* — Tyrosine. *11* — γ-Aminobutyric acid. *12* — Methionine + valine. *13* — Phenylalanine + leucine. *14* — Proline. *15* — ? *16* — Histidine. *17* — Arginine. *18* — ? *19* — Lysine. *20* — Ornithine. *21* — ? *22* — ?

— 17 and *Tussilago farfara* — 16 spots. 18 FAA were identified and 4 spots remained unidentified.

The largest amounts of FAA were found in *Cornus mas* and *Anemone nemorosa* flowers and the smallest ones in flowers of *Primula acaulis* and *Tussilago farfara,* while medium contents of FAA were recorded from flowers of *Corydalis solida* and *Bellis perennis.* In flowers of all species, the quantitatively dominant amino acids were alanine, γ-aminobutyric acid, glycine and serine. The *Cornus mas* and *Anemone nemorosa* flowers contained much aspartic acid, glutamic acid, asparagine and threonine. Arginine was present in the flowers of all species, but the *Cornus mas* flowers contained it in double and triple amounts. Large quantities of lysine were recorded from flowers of *Bellis perennis* and *Anemone nemorosa.*

On the chromatogram of the *Corydalis solida* flower extract, an intensely blue-violet coloured spot (No. 15) appeared near that of proline. It seems that the unidentified ninhydrin-positive compound producing this spot is characteristic for *Corydalis solida.* A similar, but very weak spot also appeared on chromatograms of the *Cornus mas* and *Anemone nemorosa* flower extracts. However, its violet colour suggests that it was produced by a different ninhydrin-positive compound.

The content of FAA in flowers of *Bellis perennis* and *Tussilago farfara* (which belong to the same family, *Asteraceae)* was qualitatively similar, but the flowers of *Bellis perennis* contained larger quantities of γ-aminobutyric acid, alanine and serine. The proline content was higher in the flowers of these two species than in those of the other four.

The two-dimensional chromatograms of PAA showed the presence of 16 amino acids: cysteic acid, aspartic acid, glutamic acid, serine, glycine, threonine, alanine, tyrosine, phenylalanine, leucine, methionine, valine, proline, arginine, lysine and ornithine. This PAA pattern was very similar, both qualitatively and quantitatively, in the flowers of all studied species. Only the aspartic acid, glutamic acid and threonine showed evident quantitative, species-dependent differences. The pattern of PAA obtained from the hydrolyzates of proteins solved in decoctions appeared to be more uniform, due probably to the fact that by boiling the same proteic fractions were solved.

Our results are generally in good agreement with those obtained by other authors [1, 2, 4, 5, 12] who studied the FAA in nectar, pollen, flowers and anthers from different plants and revealed species-dependent differences. With *Salvia horminum* [7] and *Corydalis solida,* the chromatographic analysis has proved the existence of an animo acid that seems to be specific. Arginine, found in large quantities in the pollen of other species [3], was present in small amounts in the flowers of species studied by us. The number of FAA determined in our study is greater than that found in some species of *Boraginaceae* [6].

The FAA pattern registered in the present study shows, in comparison with that obtained from flowers of other medicinal plants [9—11], that the amount of FAA is smaller in the vernal plants than in those blooming later. The flowers of *Matricaria chamomilla, Sambu-*

cus nigra, Robinia pseudoacacia, Hypericum perforatum, Capsella bursa-pastoris, Syringa vulgaris, Narcissus poeticus contain large quantities of FAA.

The quantity of FAA is larger, glutamic acid, serine, glycine, phenylalanine, leucine and especially threonine are present in obviously larger amounts in *Primula officinalis* than in *Primula acaulis*. At the same time, the FAA content in these species is smaller than in macromycetes [8].

Taking into consideration that the plants analyzed in the present study bloom early in the spring when the bees and other insects are in great need of pollen, the ecological role of these plants is obvious.

Conclusions. 1. The FAA content in flowers of the 6 vernal plants studied presents qualitative and mainly quantitative species-dependent differences. The total amount of FAA is evidently higher in flowers of *Cornus mas* and *Anemone nemorosa* than in those of *Primula acaulis* and *Tussilago farfara*.

2. The spot of an unidentified ninhydrin-positive compound appearing near the proline spot on the chromatogram of the *Corydalis solida* flower extract seems to be characteristic for this plant species.

3. The quantity of PAA shows small differences from one species to another.

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STUDIA UNIV. BABEŞ—BOLYAI, BIOLOGIA, XXXI, 2, 1986

THE EFFECT OF INCREASING DOSES OF METHYLCHLOR ON SUCCESSIVE GENERATIONS OF *DROSOPHILA MELANOGASTER*

NICOLAE COMAN* and MANUELA DORDEA*

SUMMARY. — The induction of resistance to methylchlor in 28 successive generations of *Drosophila melanogaster* was studied. For the same dose, the number of individuals increases exponentially from one generation to another. A sharp decrease in the number of individuals was evident with increase of the dose. At the highest concentrations, the re-establishment of population density, although significant, is rather slow. One may conclude that methylchlor induces resistance during successive generations, the effect being similar to that of its analogue, DDT.

The advantages of pesticides in fighting against species injurious for agriculture are unquestionable. Yearly, agricultural crops are attacked by about 10,000 species of injurious insects, 600 species of weeds and 1,500 diseases produced by viruses, bacteria and fungi. Non-applying of pesticides would lead to significant damages of agricultural crops. However, the utilization of pesticides in agriculture implies the precise knowledge of their way of action.

For having an effect only upon the injurious species or parasites against which the pesticide is used, it has to fulfil certain requirements such as low remanence, high specificity, lack of mutagenic action, lack of resistance-inducing effect, etc. The present and future researches aim at finding substances with such qualities.

DDT, the insecticide properties of which were discovered in 1939, had an indubitable advantage for mankind. But, as time passed by, the side effects appeared more and more evidently. The high remanence of DDT led to its accumulation in the top links of many trophic chains, followed by the decrease of viability and the destruction of entire populations. Even the penguins from the Antarctic were shown to accumulate the pesticide in their bodies. All these imposed the ceasing of production and utilization of DDT all over the world [4].

There were attempts to obtain analogues of this pesticide, having a higher specificity and a lower remanence. Methylchlor [2,2-bis(*p*-methylphenyl)-1,1,1-trichloroethane] is one of them. The effect of methylchlor at cellular level consists in diminishing the nuclear DNA content and in reversible blocking of the mitotic cycle in the G_1-phase [2].

The present paper reveals the induction of resistance to methylchlor in 28 successive generations of *Drosophila melanogaster*.

Material and methods. We used a wild strain of *Drosophila melanogaster*, from Riverside, California, reared on a "White medium", with semolina, at 25°C. Each of the 28 generations, which were analyzed, consisted of 10 vials for the

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control, each containing 10 pairs of flies. After 5 days the parents were discarded and the offspring was counted on the 12th and 14th day. The same procedure was applied for the flies submitted to the action of insecticide, which, after being dissolved in acetone, was included into the medium (at 40°C).

At the beginning, we established the DL_{50} as corresponding to 0.15% insecticide included into the medium. The flies from the successive generations were reared on a medium with higher concentrations of methylchlor than DL_{50}, namely: 0.20% for the 1st—8th, 0.22% for the 9th—13th, 0.24% for the 14th—18th, 0.26% for the 19th—23rd and 0.28% methylchlor for the last generations (24th—28th).

Results and discussions. The results are presented graphically in Fig. 1, as the mean number of offspring per vial, for both control and treated flies. As can be seen the mean number of control offspring per vial is maintained at about 126. With treated flies, the number of offspring rises from one successive generation to another, for the same dose, following an exponential curve. Every increase of the insecticide dose causes a sharp decrease in the number of flies. Afterwards, the number of offspring increases from one successive generation to another. Thus, in the 8th generation the mean number of flies per vial reached the value of 80, higher than that for DL_{50}. In the 9th generation (the first with 0.22% methylchlor in the medium) the mean number was 10, but rose to 109 in the 13th generation. Also, in the 14th generation (the first with 0.24% methylchlor in the medium) the mean number of flies decreased to 11 and in the 18th rose again, to 98.5. At higher doses of methylchlor (0.26 and 0.28%) the re-establishment of population density, although significant, is rather slow.

Our results prove that methylchlor induces resistance during successive generations. The resistance is probably the effect of the capacity

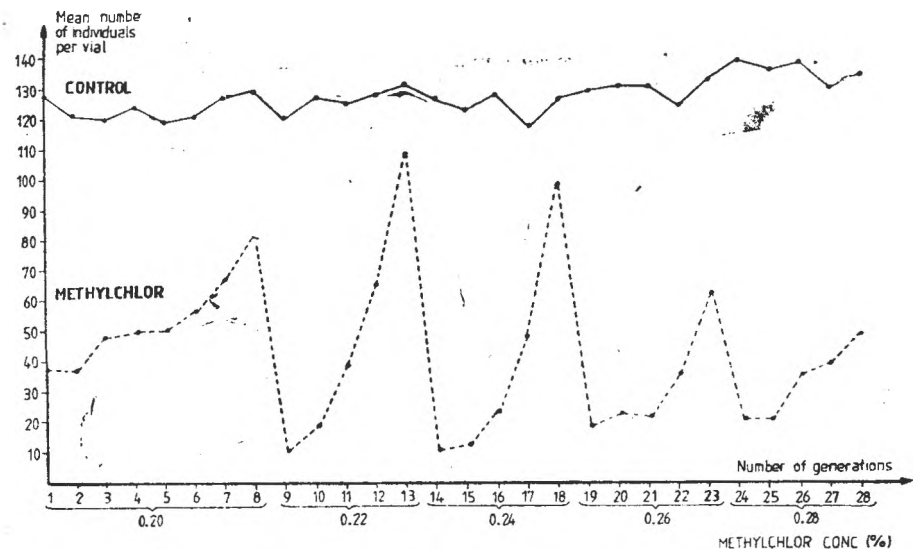

Fig. 1. *Effect of methylchlor on successive generations of* Drosophila melanogaster.

of flies to neutralize or metabolize the pesticide, which acts as a strong selective factor, with the increase of its concentration in the medium. That means that the pesticide loses its efficiency from one successive generation to another, a fact which requires either an increase of the treatment dose (followed by other disadvantages) or its replace in order to have the desired effect.

Our results are in agreement with those of other authors [1, 3], who noticed the induction of resistance at increasing doses of dipterex in *Musca domestica*. Taking into account the findings by Shepansky *et al.* [5], who established the induction of resistance in *Drosophila melanogaster* treated with DDT, it results that, in this respect, methylchlor has similar effects.

Conclusions. 1. Treatment of 28 successive generations of *Drosophila melanogaster* with methylchlor, a DDT analogue, resulted in induction of resistance to this insecticide.

2. The capacity to re-establish population density decreased with increasing methylchlor concentrations.

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EFFECTS OF BOICIL ON RESPIRATION-DEPENDENT PARAMETERS AND ULTRASTRUCTURE OF ISOLATED RAT LIVER MITOCHONDRIA

CORNELIU TARBA*, CONSTANTIN CRĂCIUN*, VERONICA CRĂCIUN* and IOAN PETRESCU*

SUMMARY. — The effects of various concentrations of Boicil (an anaesthetic drug with antirheumatic properties) on the respiratory rates, respiratory control ratio, membrane potential and the ultrastructure of rat liver mitochondria were tested by oxigraphic, spectrophotometric and electron microscopic studies.

Concentrations up to 0.02% have no significant effects on the parameters tested, regardless of the respiratory substrate used (glutamate + malate or succinate). At 0.2% Boicil, a differential effect can be observed: whereas the respiration with glutamate + malate and especially the state 3 is stimulated, only a slight stimulation of the state 4 accompanied by a moderate inhibition of the state 3 is observed with succinate (*i.e.*, a slight uncoupling effect). If 2% Boicil is used, negative effects prevail with both types of substrates, but even in this case the decrease of the respiratory control ratio is larger with succinate.

Membrane potential elicited by succinate respiration and its stability decrease as the concentration of the drug increases.

At high concentrations, electron microscopy reveals morphological changes indicative of a functional impairment of mitochondria. However, in the case of glutamate + malate these changes are moderate, as deduced from a relatively small number of supercondensed and falciform mitochondria, whereas in the case of the succinate about half of the mitochondria are swollen and the rest are condensed or supercondensed.

It is concluded that Boicil might exert its antirheumatic effect through the heat released as a consequence of the concomitant stimulation of NADH-dependent respiration and of the slight uncoupling effect that probably occur at the therapeutic concentrations.

Boicil, which is an ethanolic extract from *Helleborus* belongs to the group of local anaesthetics, having analgesic and antiinflamatory properties. It has been suggested that this drug exerts both an action at the level of the cell membrane, resulting in a change of membrane excitability or (more generally) permeability [1, 5] and an inhibitory effect on the bioenergetic properties of the cell, more specifically on the function of mitochondrial enzymes [5, 11].

Considering our experience regarding the effect of certain anaesthetic drugs, such as procaine and procaine-based preparations, on the function and ultrastructure of rat liver mitochondria [10], we decided to undertake a relatively complex study of the effects of Boicil on the mitochondrial respiration and several respiration-dependent parameters (respi-

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ratory control ratio, phosphorylation and membrane potential) in correlation with the ultrastructural aspects of the mitochondrial morphology [9]. Respiration and phosphorylation were selected for their fundamental importance in the process of energy production and conservation (see for ex. [4]) and membrane potential for being the high energy intermediate between oxidation and phosphorylation [6]. Electron microscopy served as a direct proof of the effects of Boicil on the mitochondrial structure.

Materials and methods. Mitochondria were obtained by standard procedures [3] from the liver of white female rats (150—200 g). The isolation medium consisted of 250 mM sucrose, 10 mM Tris pH 7.3 and 0.1 mM EDTA, whereas the washing and suspending medium lacked EDTA. Preparations were performed at 0—4°C and the rest of the operations at the room temperature.

Respiration rates were measured in a 0.5 ml cell with a Clark oxygen electrode (Yellow Springs, Ohio) connected to a LP7e polarograph and corresponding recorder (Laboratorni Pristroje, Prague). The medium used for these measurements, subsequently referred to as phosphorylation medium (PM), consisted of 100 mM sucrose, 75 mM KCl, 100 mM Tris pH 7.3, 5 mM KP_i (P_i = inorganic phosphate), 2 mM $MgSO_4$ and 0.5 mM EDTA.

Concentrated Boicil (Boicil forte) was added directly to the oxigraphic cell or spectrophotometric cuvette containing the suspending medium so as to achieve relative concentrations (volume/volume) of 0.02%, 0.2% and 2%. Higher concentrations were not used in order to avoid possible anaesthetic effects of the ethanol itself. Substrates (glutamate + malate or succinate) were added in the concentration of 10 mM each. Mitochondria (1 mg/ml with succinate or 2 mg/ml with glutamate + malate) were injected through the stopper capillary and after 1—2 min the respiration was stimulated by a pulse of 0.2 mM ADP. The ADP injection was repeated one or two more times at 2—3 min intervals and respiration rate (RR), for each state, respiratory control ratio (RCR) and the ratio of ADP to oxygen (ADP/O) were calculated for each ADP addition from the oxigraphic traces. Statistical calculations on the oxigraphic results were performed as previously described [10].

Membrane potential was estimated by the use of 3,3'-diethylthiadicarbocyanine, a cyanine dye usually abbreviated as diS—C_2-(5), according to a principle described at length elsewhere [7, 8]. Its absorbance change at 660 nm was recorded with a Specord M 40 spectrophotometer (Carl Zeiss, Jena), to which certain modifications were applied so as to allow kinetic recordings with repetitive additions. The basic medium (BM) used for membrane potential was similar to the PM medium but it was devoid of KP_i and EDTA. The exact conditions and the additions made in each case are described in the corresponding figure legend.

The ultrastructure was studied with the aid of a TESLA BS-500 electron microscope. For electron microscopic preparations, a 0.1—0.2 ml aliquot was extracted from the oxigraphic cell after the consumption of the second ADP pulse. This was injected into a 0.6 ml microtube containing 0.3—0.4 ml prefixing medium (1% glutaraldehyde solution in 150 mM phosphate buffer, pH 7.4). Mitochondria were then sedimented by centrifugation at 7000 *g*, for 15 min. The pellet obtained was processed according to current techniques for electron microscopy.

All the chemicals used were of analytical grade. ADP and rotenone were from Sigma and diS—C_2-(5) from Eastman-Kodak. Boicil forte is a Romanian product obtained from the Drug Enterprises, Bucharest.

Results and discussion. Table 1, which contains the results of the measurements of the respiratory parameters in the absence and presence of different concentrations of Boicil, enables us to make certain useful observations with regard to the effects of this drug.

Table 1

Respiration rates (RR) (ng oxygen atoms/min. mg protein) and the respiratory control ratios (RCR) in mitochondria treated with different concentrations of Boicil

Substrate	Treatment (Group) and number of preparations	RR_1		$RCR_1 \pm SEM$	RR_2		$RCR_2 \pm SEM$	RR_3		$RCR_3 \pm SEM$
		State 3	State 4		State 3	State 4		State 3	State 4	
Glutamate + Malate	0.02% BOICIL 2	66.0	15.0	4.40±0.05	70.5	15.0	4.70±0.05	71.5	14.0	5.10±0.10
	CONTROL 2	58.5	15.0	3.90±0.28	68.0	15.0	4.50±0.24	74.0	14.5	5.10±0.10
	0.2% BOICIL 2	85.0*	18.0	4.70±0.05*	87.0*	18.5*	4.70±0.10	90.0*	18.0*	5.00±0.07
	CONTROL 3	65.0	16.0	4.05±0.18	75.0	15.5	4.80±0.17	74.5	15.0	4.95±0.08
	2% BOICIL 2	76.0	20.5*	3.70±0.05*	78.0	19.5*	4.00±0.08*	80.0	19.0*	4.20±0.10*
Succinate	0.02% BOICIL 2	79.0	29.0	2.70±0.05	91.0	25.0	3.65±0.10	Not determined		
	CONTROL 2	80.0	29.0	2.75±0.05	91.0	27.0	3.35±0.20			
	0.2% BOICIL 2	73.5	32.0	2.30±0.23*	79.0*	27.0	2.90±0.15*			
	2% BOICIL 2	96.0*	46.5**	2.05±0.05**	108.0*	42.5**	2.55±0.17**			
	CONTROL 2	109.0	37.0	2.95±0.10	135.0	35.0	3.85±0.18			

* Significant difference ($p < 0.05$).
** Highly significant difference ($p < 0.01$).

With *glutamate + malate,* in the presence of 0.02% Boicil, although the first two ratios of the respiratory control (RCR_1 and RCR_2) are larger than in the corresponding control, the differences are not significant. Electron microscopy (Fig. 2) also does not indicate visible differences as compared to the control (Fig. 1). In both cases the picture is dominated by slightly condensed mitochondria and very few orthodox and supercondensed forms. This terminology refers to the aspect of the electron micrographs and was introduced by Hackenbrock [2]. Mitochondria *in situ* exhibit the orthodox configuration, with expanded matrix, visible cristae and little electron density. Depending on their state of energization and the conditions of isolation, well prepared mitochondria can exhibit both the orthodox and the condensed form (contracted matrix of high electron density in contrast to small spaces devoid of electron density).

At 0.2% Boicil, significant differences begin to appear in the magnitude of state 3 respiration. The first respiratory control ratio (RCR_1) is also significantly increased. The rest of the ratios are not larger because the increase in state 3 is paralleled by an increase in state 4, the ratio of the two (RCR) remaining constant.

If concentration is increased to 2%, the stimulating effect of Boicil on state 3 decreases whereas the rate of state 4 continues to increase. This leads to a significant decrease of the RCR. The electron microscopic images (Fig. 3) show a moderate increase in the percentage of supercondensed and falciform mitochondria. Supercondensation is a negative and almost surely irreversible phenomenon, which finally leads to membrane disruption and matrix disintegration.

With *succinate* (see Table 1), there are no significant differences at 0.02% Boicil. Electron microscopic images (not shown at this concentration) are similar to those of the control (Fig. 4). However, contrary to the case of glutamate + malate, 0.2% Boicil has negative effects on the respiration. Thus, at the second ADP injection the magnitude of the state 3 decreases and, as a consequence, RCR_2 becomes significantly lower than in the corresponding control.

At 2% Boicil the effects on the respiration are spectacular: a very significant decrease of the respiratory control ratios, occurring on the basis of a state 3 decrease and a state 4 increase (concomitant inhibition and uncoupling of oxidative phosphorylation). A very peculiar aspect is presented by electron microscopy. Beside supercondensed (falciform) mitochondria, the image in Fig. 5 shows many mitochondria in a state that bears a vague resemblance to the orthodox state. At a closer examination one can see that these mitochondria are, in fact, in a swollen state, almost completely devoid of electron density. They have lost their osmotic barrier and are on the irrevesible way of disruption. Identical aspects can be seen in mitochondria treated with certain antihyperlipidemic drugs [12], which also have negative effects on mitochondrial functions, similar to those described for Boicil (especially uncoupling effect).

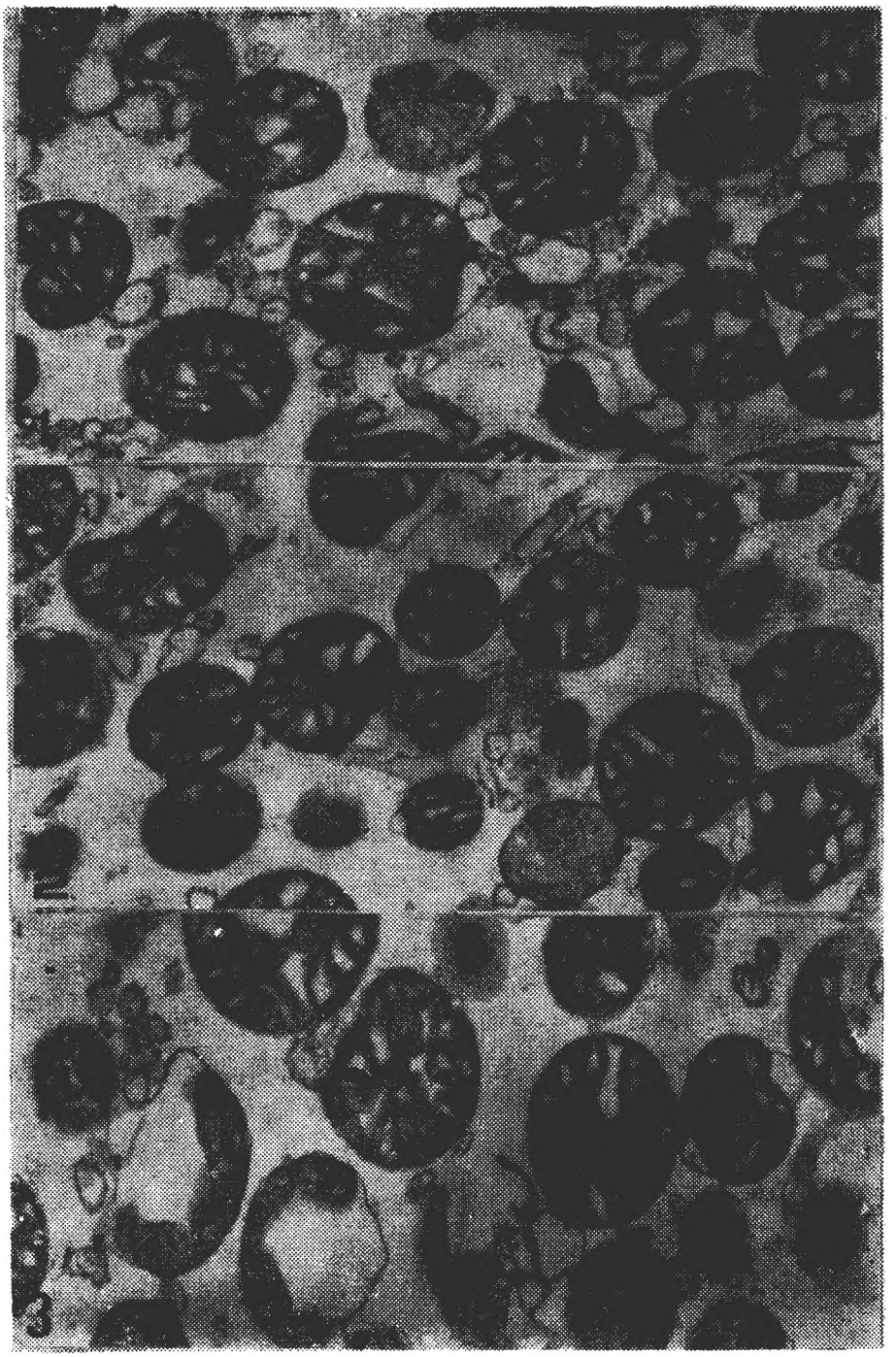

Fig. 1. *Ultrastructural aspects of control mitocondria respiring with glutamate + malate,* 22,000X
Fig. 2. *Ultrastructural aspects of mitochondria respiring with glutamate + malate and treated with 0.02% Boicil.* 22,000X.
Fig. 3. *Effects of 2% Boicil on the ultrastructure of mitochondria respiring with glutamate + malate.* 28,800X.

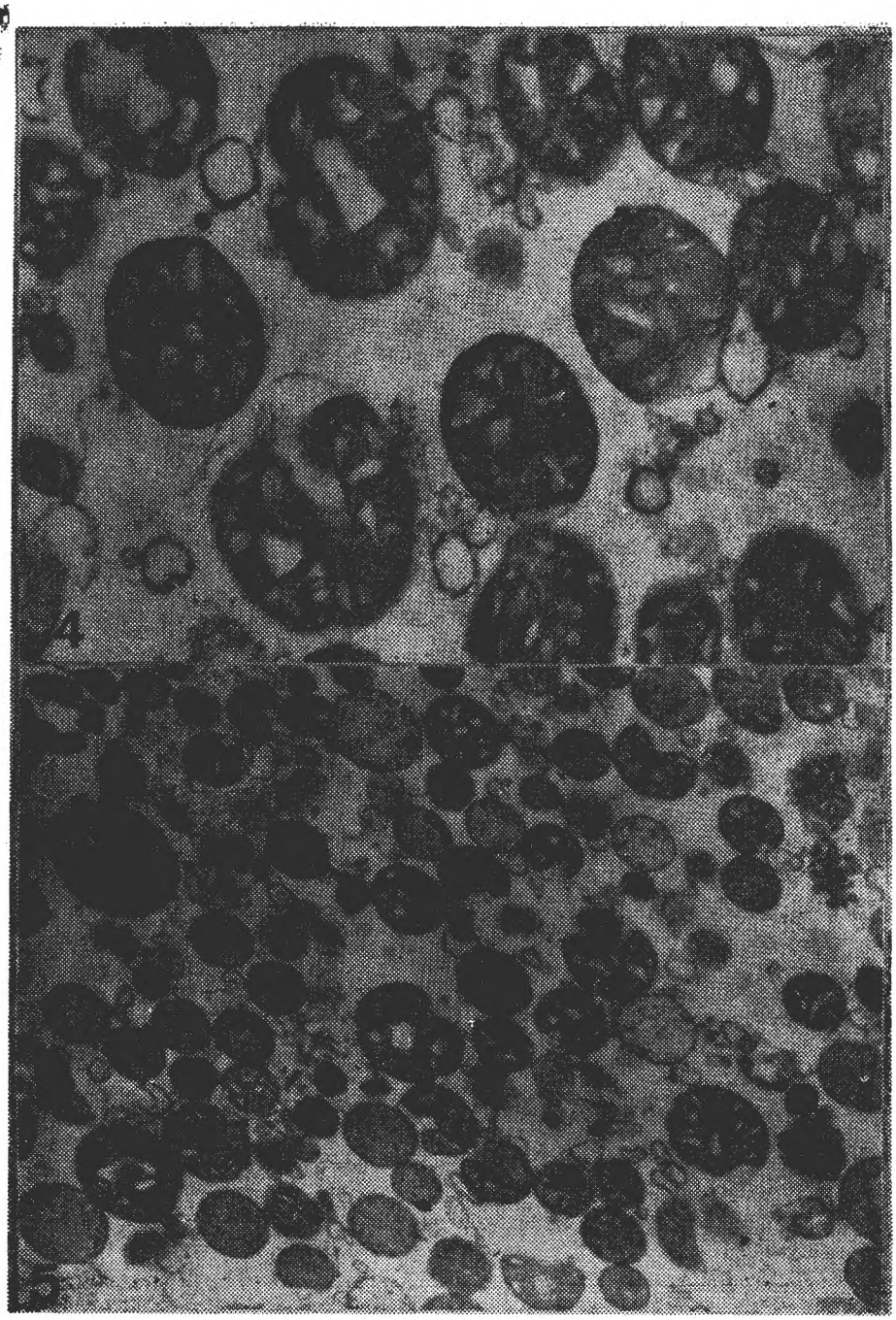

Fig. 4. *Ultrastructural aspects of control mitochondria respiring with succinate.* 28,800X.
Fig. 5. *Effects of 2% Boicil on the ultrastructure of mitochondria respiring with succinate.* 12,000X

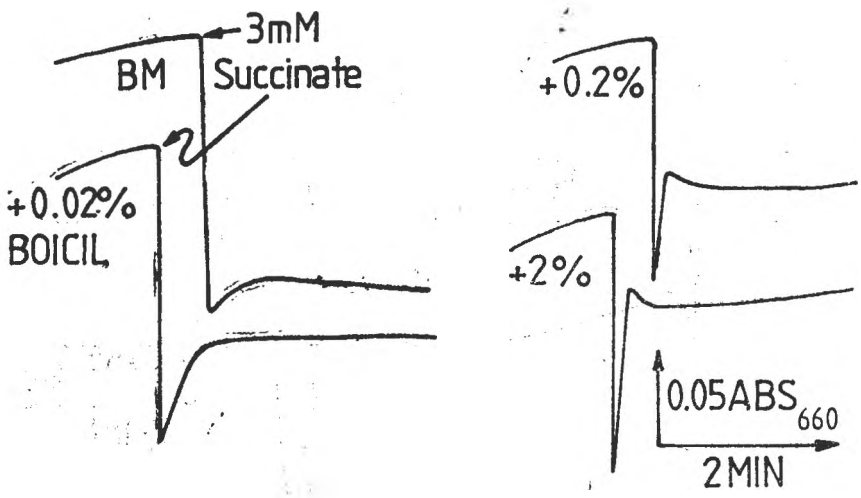

Fig. 6. *Effect of different concentrations of Boicil on the membrane potential elicited by succinate respiration.*
0.75 mg/ml mitochondrial protein in the basic medium (BM), 5 μM rotenone and 2.5 μM diS$-C_2-$(5).

Due to residual NADH-dependent respiration it is very difficult to study membrane potentials with glutamate + malate. Usually, in order to illustrate membrane potential characteristics (magnitude and kinetics), one inhibits this residual respiration with rotenone and energizes mitochondria with succinate. Such recordings are shown in Fig. 6. It can be seen that, in the absence of EDTA, increasing concentrations of Boicil decrease the magnitude and the stability of the membrane potential. Even in the presence of EDTA (which, by chelating Ca^{2+}, usually prevents the easy uncoupling of mitochondria), the membrane potential exhibits a very peculiar aspect (Fig. 7). After an initial transitory increase, it undergoes a series of oscillations and stabilizes at a low value. This clearly demonstrates the inability of mitochondria to withstand even slight metabolic efforts, a fact which is in full agreement with the other results of this study.

Although we could not find any significant difference in the value of ADP/O ratio, with neither of the substrates, judging by the uncoupling effect and the small magnitude of the membrane potential, it is clear that the efficiency of phosphorylation must have been affected also.

Conclusions. The results obtained with the two types of substrates tend to indicate a differential effect of Boicil. Beside its general uncoupling effect, at low concentrations, Boicil has a stimulating effect on the NADH-dependent respiration, whereas higher concentrations clearly indicate a negative effect and a greater sensitivity of the succinate respiration to this drug. This complex behaviour may explain in part its antirheumatic effect, which could be achieved through the heat

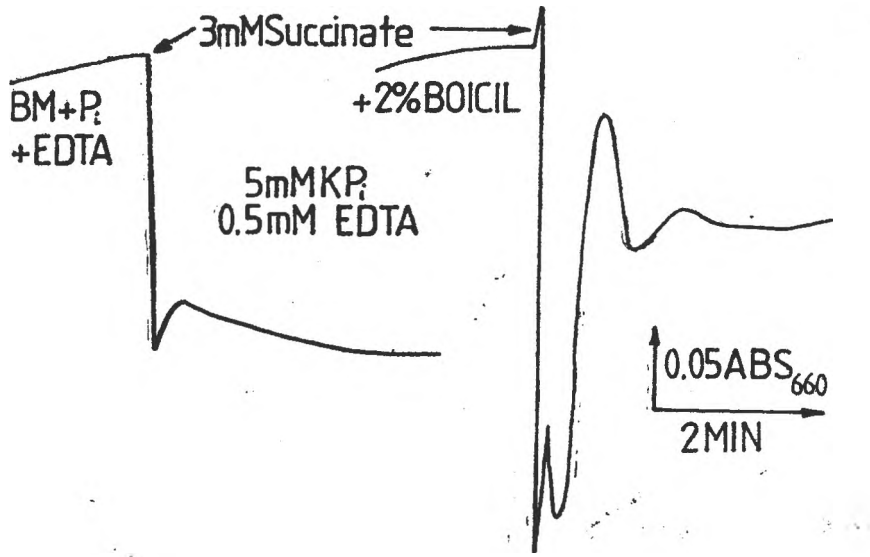

Fig. 7. *Characteristics of membrane potential in the presence of P_i, EDTA and 2% Boicil*
Other conditions as in Fig. 6.

released as a consequence of the parallel stimulation of the NADH-dependent respiration and the slight uncoupling effect, probably present even at the therapeutic concentrations.

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COMPARATIVE TOXICOLOGICAL STUDY OF TWO ROMANIAN SYNTHETIC PYRETHROIDS

GHEORGHE FRECUŞ*

SUMMARY. — The toxicological study of two Romanian synthetic pyrethroids — phenothrin and phenvalerate — underlined that, like the natural pyrethroids, they are more toxic for insects than for mammals. The toxicity of these substances is imprinted especially by the 3-phenoxybenzyl residue present in their molecules. Taking into account that the DL_{50} of the phenothrin is very low in insects and very high in mammals, this pyrethroid is recommended to be preferentially used as compared to the phenvalerate.

This work aims at dealing with two main aspects of the biology of synthetic pyrethroids: 1. the accurate knowledge of the relation between the molecular structure and the action mechanism of synthetic pyrethroids, and 2. the option for a synthetic pyrethroid that should be highly toxic for insects and only slightly for mammals.

Two synthetic pyrethroids were tested: phenothrin [3-phenoxybenzyl (+) *cis-trans*-dimethyl-(2,2-dimethylvinyl)-cyclopropane-1-carboxyl], and phenvalerate [α-isopropyl-4-chlorophenyl acetic acid 3-phenoxy-α-cyanobenzyl ester] (Fig. 1).

The parameters determined were: 1. embryotoxic and teratogenic effects, 2. cytogenetic effects, 3. acute toxicity (DL_{50}) and subchronic toxicity following a 6-month treatment with daily doses of 1 and 5% DL_{50}.

Material and methods. Experiments were conducted on white female Wistar rats. Embryotoxic and teratogenic effects were tested on female rats weighing about 70 g each. Cytogenetic effects were observed by analyzing chromosome aberrations and the appearance of micronuclei. Acute toxicity (DL_{50}) was determined on female rats weighing 150—180 g. The resulting data were processed by using the method of probits [2], according to the graphic and numerical procedure described by W e b e r [8]. Subchronic toxicity was studied on female white rats that were 30 days old when the treatment started. Experiments were conducted for 6 months, pyrethroids being administered daily in doses of 1 and 5% DL_{50}, added to the food.

Both the above-mentioned tests and the analysis of the metabolic indices presented in Figs. 2—5 were performed by usual clinical techniques [6], excepting

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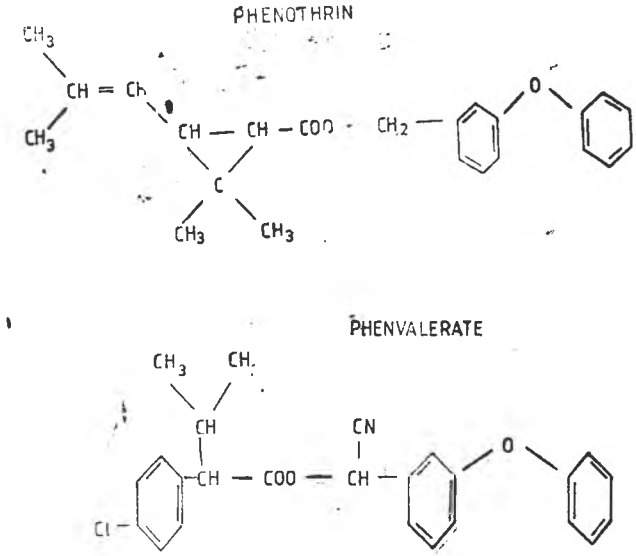

Fig. 1. *Chemical structure of the studied pyrethroids.*

glycogen, ascorbic acid and total thymus and liver lipid contents, for the determination of which we applied the methods described by Montgomery [7], Asatiani [1] and Folch *et al.* [3], respectively.

Results and discussion. DL_{50} was 20,000 mg/kg body weight for phenothrin and 1,326 mg/kg body weight for phenvalerate. None of these two Romanian pyrethroids caused obvious embryotoxic and/or teratogenic effects. Nevertheless, when compared to control, there could be noticed a 5% decrease in weight and a 8% decrease in length of foetuses under the influence of phenothrin, and a 31% decrease in weight and a 11% decrease in length of foetuses under the influence of phenvalerate. This shows that these insecticides have a certain toxic effect upon mammals. There were no cytogenetic effects: neither of the two pyrethroids affected the hereditary material, the chromosome aberrations varying between the normal limits of spontaneous mutations, also noticeable in control.

In Figs. 2—5 the changes are given as percentage differences compared to control. These show that the two pyrethroids caused a decrease in glycaemia, haemoglobin content and the activity of two blood transaminases. A decrease in liver and thymus protein content as well as a change in the activity of some liver transaminases and a decrease

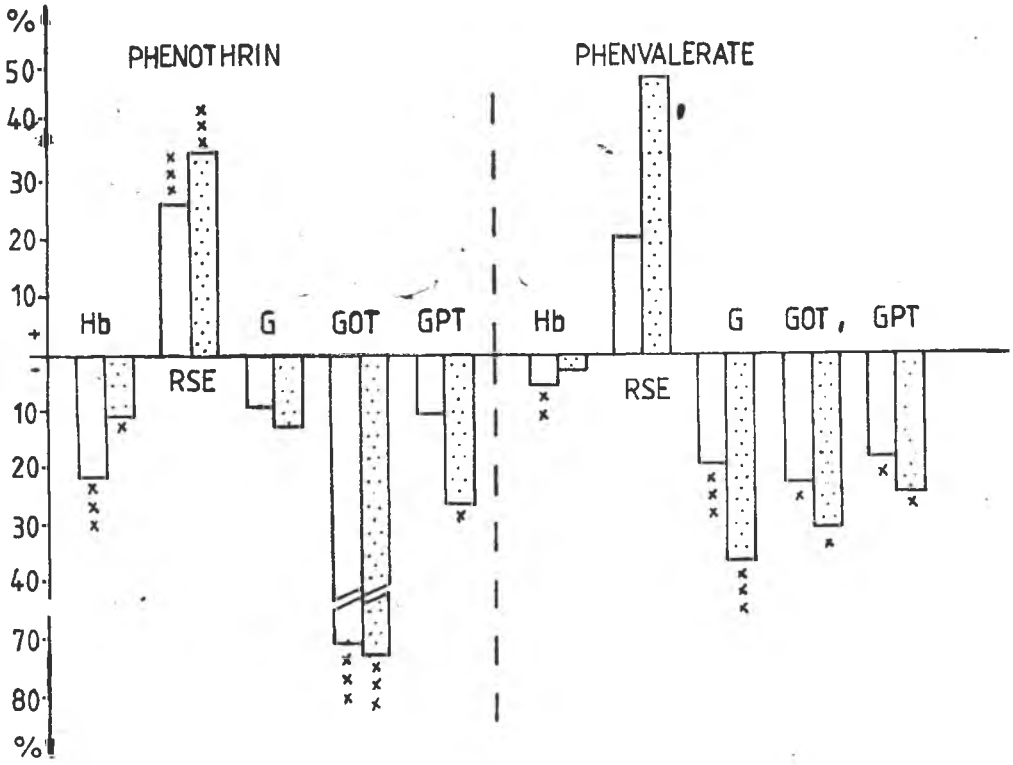

Fig. 2. *Changes induced by phenothrin and phenvalerate in the blood.*
White columns — Percentage differences induced by daily doses of 1% DL_{50} as compared to the control. *Dotted columns* — Similar changes induced by daily doses of 5% DL_{50}. *Hb* — Haemoglobin. *RSE* — Rate of sedimentation of erythrocytes. *G* — Glycaemia. *GOT* — Glutamic-oxaloacetic transaminase activity. *GPT* — Glutamic-pyruvic transaminase activity. Statistical significance: x — $p < 0.05$, xx — $p < 0.01$, xxx — $p < 0.001$.

in weight of the thymus can also be noticed. The corroboration of these data with those concerning the weight and length of foetuses leads to the conclusion that protein metabolism is undoubtedly altered. Some alterations of the carbohydrate consituents in blood, liver, thymus and adrenal glands are also obvious.

These modifications are considered to be toxic effects of the complex structures of the pyrethroids and taking into account literature data [4, 5], it seems that the 3-phenoxybenzyl residue is the toxic element of the two insecticides.

It is known [4] that 3-phenoxybenzoic acid forms liposoluble compounds in the body of mammals rather than hydrosoluble ones, which may explain the metabolic alterations noticed. The same authors [4]

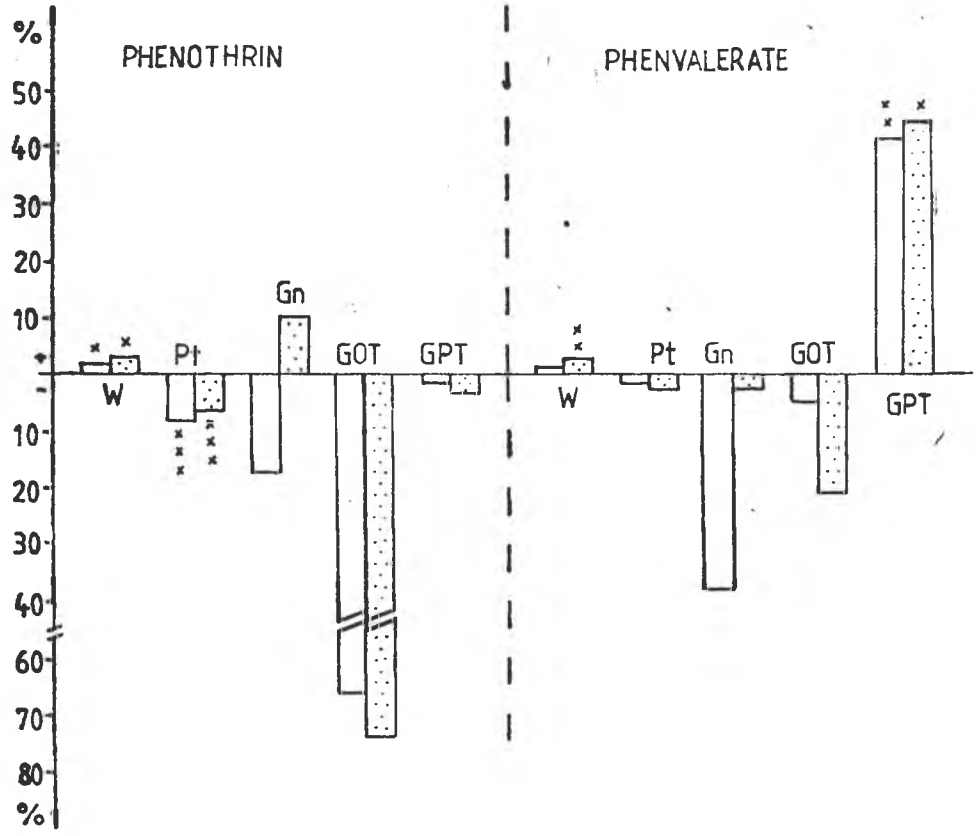

Fig. 3. *Hepatic changes induced by phenothrin and phenvalerate.*
W — Water content. *Pt* — Total protein content. *Gn* — Glycogen content. For other explanations see Fig. 2.

also showed that the 3-phenoxybenzoic acid administered to rats *per os* brought about the incorporation of 3-phenoxybenzoyl dipalmitin into the skin, and in rat, dog and rabbit liver slices the same acid caused the formation of 3-phenoxybenzoyl diacyl glycerols containing oleic and stearic acids. Figs. 2—5 also reveal the parallelism of the metabolic changes of 11 indices out of 19 (*i.e.* 58% of the indices), a fact which can be ascribed to the existence of 3-phenoxybenzyl residue within the structure of the studied pyrethroids.

Conclusions. 1. The toxic character of the two pyrethroids studied can be ascribed to their 3-phenoxybenzyl residue. 2. Since phenothrin is much less toxic for mammals than phenvalerate, the former should be preferred in controlling pest insects.

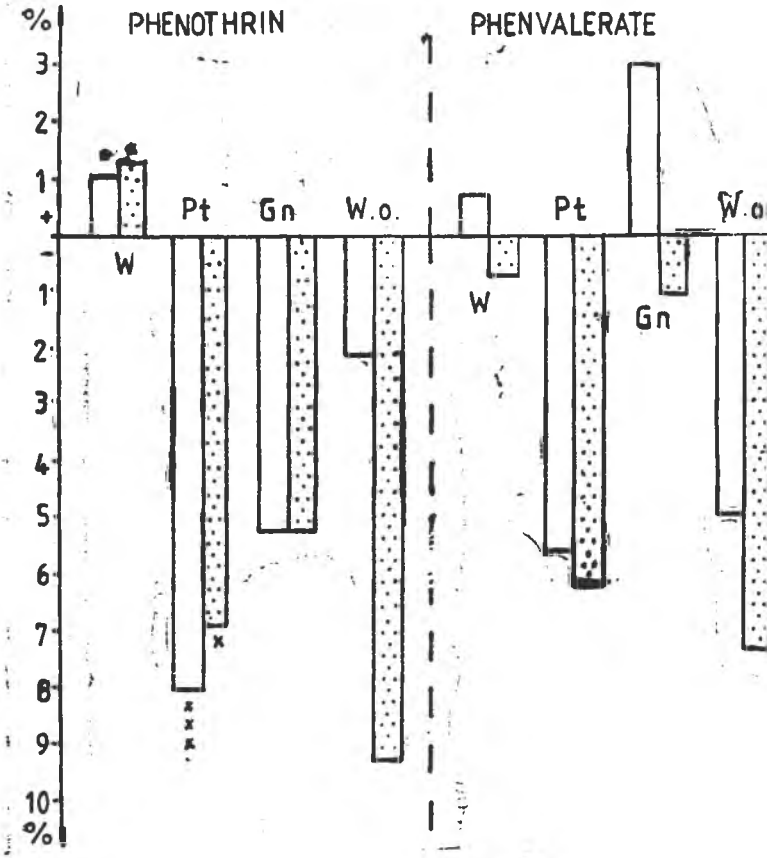

Fig. 4. *Changes induced by phenothrin and phenvalerate in thymus.* *W.o.* — Weight of organ. For other explanations see Figs. 2—3.

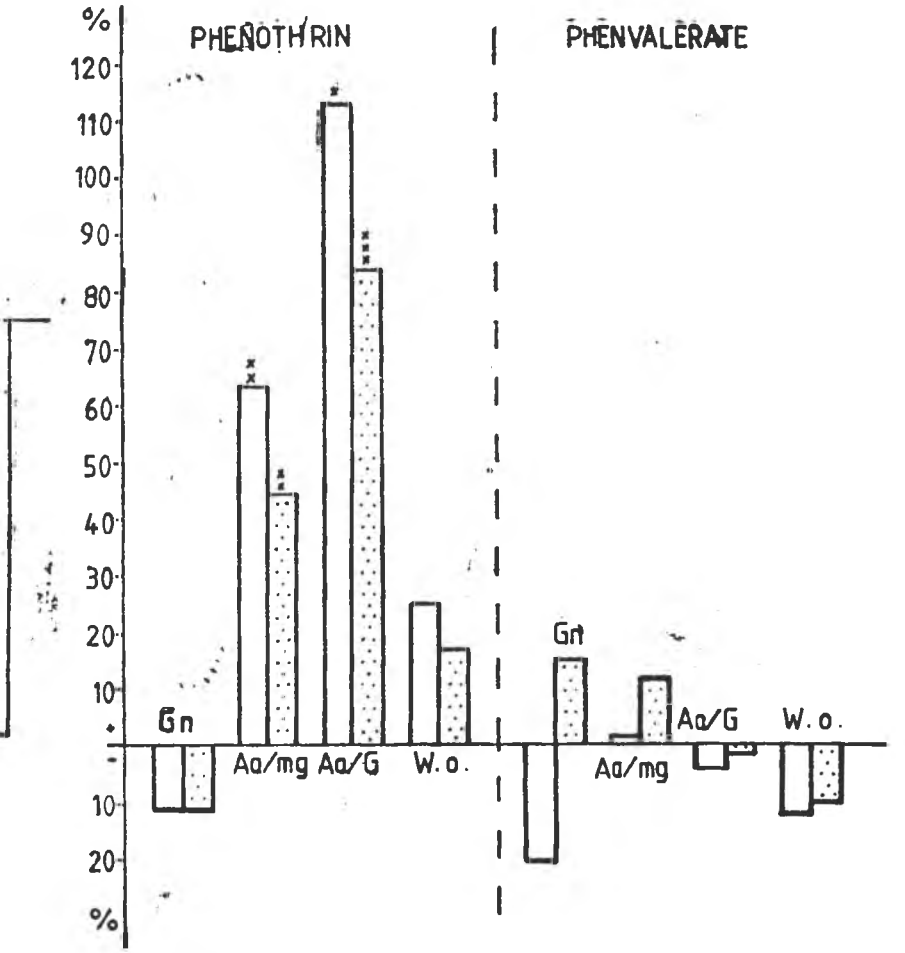

Fig. 5. *Changes induced by phenothrin and phenvalerate in adrenal glands.* *Aa/mg* — Ascorbic acid/mg gland. *Aa/G* — Asorbic acid/whole gland. For other explanations see Figs. 2—4.

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RECENZII

Advanced Molecular Genetics, Edited by A. Pühler (FRG) and K. N. Timmis (Switzerland), Springer-Verlag, Berlin, Heildelberg, New York, Tokyo, 1984. IX + 347 pages with 98 figures and 20 tables.

The book comprises 8 chapters (Basic methods, Mutagenesis, Gene cloning, Gene expression, DNA sequencing, Electron microscopy, Transcription and DNA replication) and represents a comprehensive study of molecular genetics, containing an impressive amount of data. The purpose of this manual is to present most of the important methods of molecular genetics, in a series of simple experiments, many of which can be accomplished by biochemists, microbiologists or biotechnologists that have only limited experience in genetics. The remainder of the experiments require either a closer familiarity with the subject, or the guidance of someone with great experience. It should be noted that plasmids are the experimental subjects in many of the protocols presented in this manual.

The volume represents a rich source of bibliographical information referring to new laboratory techniques in molecular genetics.

This book is recommended, therefore, not only to enable researchers to apply new procedures for ongoing projects, but it also serves as a basis for teaching molecular genetics techniques in laboratory courses. The majority of protocols in this manual (as the Editors specify) were developed for and tried and tested in laboratory courses organized under the auspices of the European Molecular Biology Organization.

NICOLAE COMAN

Population Biology and Evolution, Edited by K. Vöhrmann (FRG) and V. Loeschcke (Denmark), Springer-Verlag, Berlin, Heidelberg, New York. Tokyo, 1984, IX + 270 pages with 74 figures and 32 tables.

This volume contains the papers presented at the Symposium on population biology held at the University of Tübingen in May 1983.

The book is divided into chapters corresponding to the 8 topics chosen. The first chapter is devoted to the relations between genotype and phenotype, and it is followed by a chapter on quantitative genetics and selection in natural populations. Chapters 3 deals with theoretical aspects of density regulation and life histories. The genetic heterogeneity and ecological factors are discussed in Chapter 4. The next chapter concerns genetic structure and demography in plant populations. Chapter 6 covers population differentiation and asexual reproduction, and is followed by contributions on theoretical aspects of coevolution. The volume concludes with some comments on models in population genetics and evolutionary ecology.

The book „Population Biology and Evolution" is an important source of bibliographical information for research workers, teachers and students in the field of population genetics and population ecology.

NICOLAE COMAN

Mutations in Man, Edited by Günter Obe (FRG), Springer-Verlag, Berlin, Heidelberg, New York, Tokyo, 1984, IX + 327 pages with 73 figures and 71 tables.

The authors reveal that the induction of mutations in human somatic cells has been detected, and there is ample evidence that some of these mutations are initiators of cancer. The authors emphasize the importance of understanding the mechanisms of mutation induction, recognizing mutagenic agents, the limit of the exposure to such mutagens and the prevention of the action of new mutagens.

The book „Mutations in Man" may be helpful in a better understanding of these problems and their solution.

The following aspects are discussed: chemical mutagens in the human environment and their detection, their reactions with cellular DNA, and the repair of DNA lesions; structure and organization of the human genome; frequencies and origin of gene or point mutations, and of chromosomal abnormalities; origin and significance of chromosomal alterations; the human lymphocyte test system, chromosomal alterations in lymphocytes of patients under chemotherapy, and of cigarette smokers; sperm anomalies in smokers and nonsmokers; estimation of the genetic risk.

This volume is a valuable source of bibliographical information for biology lecturers, researchers and students.

NICOLAE COMAN

The Chemistry of Allelopathy. Biochemical Interactions among Plants, Edited by A. C. Thompson *(La chimie de l'allélopathie. Interactions parmi les plantes,* Sous la rédaction de A. C. Thompson), American Chemical Society, Washington, D.C., 1985, 470 pages avec 96 figures et 116 tableaux.

L'allélopathie — un type de relations écophysiologiques chez les plantes se référant aux effets répressifs ou nuisibles que les végétaux manifestent les uns contre les autres (les mots grecs „allelon" + „pathos") — est aujourd'hui étudiée avec une grande insistence justifiée par ses importantes implications applicatives. Les quelques ouvrages monographiques devenus classiques (H. Molisch, 1937; G. Grümmer, 1955; E. L. Rice, 1974, etc.), ainsi que des pertinentes synthèses (C. H. Muller, 1966; R. H. Whittaker, 1970; T. Swain, 1974; A. R. Putnam et W. B. Duke, 1978, etc.), continuées par un très récent recueil de 31 travaux, sont une preuve convaincante pour l'inépuisable actualité des recherches sur l'allélopathie et pour leur avenir.

L'étude de ce phénomène biologique met en évidence la signification qu'il a dans la nature et dans les agroécosystèmes, avec ses perspectives dans la vie pratique.

Une des applications les plus importantes, peut-être, c'est le contrôle des mauvaises herbes, compte tenant du risque de l'emploi des herbicides synthétiques, incriminés comme polluants et cancérigènes.

Encore une application des interactions allélopathiques c'est le rôle considérable qu'elles jouent dans la succession de la végétation terrestre et pour le contrôle de la flore marine, par exemple le cas de *Hydrilla,* un véritable menace d'obstruction pour les voies maritimes.

Par les substances allélopathiques et à travers les mécanismes allélopathiques se passe dans la nature le réglage de la croissance et du développement des plantes et l'influence de la germination des semences, il s'exèrce un contrôle supplémentaire de la phytopathogenèse.

Les auteurs des ouvrages inclus par A. C. Thompson dans le tome que nous venons de présenter sont des noms prestigieux pour le domaine de l'allélopathie.

Quelques uns des ouvrages du recueil sont des comptes rendus généraux sur la recherches dans ce domaine et surtout sur l'allélopathie dans l'agriculture, concernant le contrôle des mauvaises herbes et de la germination des semis, l'absorption des substances minérales et l'assimilation de l'azote; d'autres travaux se réfèrent à la composition chimique et aux caractéristiques biologiques des agents allélopathiques de diverses provenances; sont encore étudiées les relations des microorganismes du sol et l'activité radiculaire des végétaux; d'une égale importance sont les travaux sur les substances allélopathiques des plantes aquatiques et des algues, ainsi que celles qui viennent de contrôler les relations hôte-parasite.

La conception fondamentale de tous les auteurs pour étudier ce phénomène naturel pour notre bénéfice est une vaste collaboration interdisciplinaire pour intégrer les découvertes réalisées.

EUGENIA CHIRCĂ et ANA FABIAN

Biogeotechnology of Metals, Edited by G. I. Karavaiko (USSR) and S. N. Groudev (Bulgaria), Centre of International Projects of the USSR State Committee for Science and Technology, Moscow, 1985, 417 pages with 112 figures and 85 tables.

Biogeotechnology emerged in the last two decades as a new branch of

geological microbiology. It is concerned with applications of microorganisms for geotechnological mining and processing of minerals, for metal extraction from solutions, and for industrial effluent treatment in the view of removing metals and protecting the environment.

„Biotechnology of Metals" comprises both lectures delivered at the International Training Course on Microbiological Leaching of Metals from Ores, held in the USSR and the People's Republic of Bulgaria on May 24 to June 20, 1982, and papers presented at the International Seminar on Modern Aspects of Microbiological Hydrometallurgy held in Moscow on June 21—26, 1982.

The volume consists of Preface, Introduction, a review by the Editors on „Biogeotechnology of metals, its history, tasks and trends of development", and 6 parts containing 27 papers elaborated by Soviet and Bulgarian scientists and also by scientists from Australia, Canada, France, India, Italy, Japan and the USA.

Part I, „Physiology and biochemistry of microorganisms important in biogeotechnology of metals", comprises 4 papers, the first of which is a review and the other three deal with some characteristics of iron-oxidizing thiobacilli isolated from uranium mine leach liquors; mathematical modelling of the growth and development of *Thiobacillus ferrooxidans* with regard to oxidation of Fe^{2+} and sulphide minerals; differences between strains of *Thiobacillus ferrooxidans* with respect to their ability to oxidize sulphide minerals.

Six papers are grouped into Part II, „Role of microorganisms in chemical element cycles". Their topics are the following: kinetics of the sulphur cycle; role of microorganisms in the migration of some elements; role of microorganisms in the hypergene migration of gold; microorganisms involved in turnover of iron and manganese: their application in hydrometallurgy; microbial leaching of metals from rocks (essentially from granitic rocks); microflora of rocks and its role in the leaching of silicate minerals.

Part III consists of three papers dealing with the „Physico-chemical principles and mechanism of bacterial oxidation of sulphide minerals".

Part IV, devoted to the „Technologies of bacterial leaching of metals from ores", includes 8 papers having the following themes: bacterial leaching of metals in tanks — non-ferrous concentrate treatment: technology and flow sheets; intensification of bacterial oxidation of iron and sulphide minerals by a *Thiobacillus ferrooxidans* culture at a high cell concentration; scientific fundamentals of technology of tank leaching of uranium; technical aspects and problems of chemical and bacterial leaching of low-grade and refractory copper ores; technology of bacterial dump leaching of metals from ores; modelling production processes in bacterial leaching; application of microbiological methods to underground leaching of uranium ores; processing of metallurgical slags and ore-dressing tailings.

Part V, entitled „Environmental protection in microbiological hydrometallurgy", consists of two papers dealing with the evaluation of acid production potential of mining waste materials and with the use of microorganisms for industrial waste water treatment, respectively.

In the first paper of Part VI, „New trends in biogeotechnology", a special attention is paid to the possibility of reducing methane concentration in the air of coal mines by using methane-oxidizing bacteria introduced in the form of artificial filter into the rock breakage zone. The other three papers of Part VI have the following topics: microbiological methods of manganese leaching in India; biodegradation of aluminosilicates: progress and perspectives; sulphate-reducing bacteria and some microscopic fungi in ore preparation and hydrometallurgy.

The volume reflects the significant progress achieved in many fields of biogeotechnology, its economic and ecological importance. It is a valuable source of information for various categories of readers including microbiologists, biotechnologists, geochemists, geotechnologists, and other specialists interested in the extraction and processing of minerals.

ŞTEFAN KISS

Microbiologie industrială şi biotehnologie, Sub redacţia: N. D. Topală *(Industrial Microbiology and Biotechnology*, Edited by N. D. Topală), Universitatea „Al. I. Cuza" Iaşi, Întreprin-

derea de Antibiotice Iaşi, Consiliul Naţional al Inginerilor şi Tehnicienilor and Societatea de Ştiinţe Biologice Filiala Iaşi, 1986, 958 pages with 368 figures and 278 tables.

The volume comprises 134 papers which were presented at the 5th Symposium on Industrial Microbiology and Biotechnology held in Iaşi on October 25—26, 1985. The Proceedings of the first four symposia were reviewed in the Studia Univ. Babeş-Bolyai, Biologia (1981, 26 (2), 73—74 and 1985, *30*, 76—77).

After a Foreword by Professor N. D. Topală, four review articles are included. We quote them: „Biotechnology in the new scientific and technical revolution" (M. Florescu); „Immobilization of enzymes, cell organelles and microbial cells. Present status and perspectives" (N. D. Topală, D. C. Cojocaru and V. G. Artenie); „Ni-Enzymes involved in the metabolism of methanogenic bacteria" (H. D. Schell); „Biomass upgrading by biotechnology and bioenergetics" (G. Muscă and R. Giurcă).

The other review articles (8) and the original papers (122) cover most topics of industrial microbiology and biotechnology as specified below.

Production of microbial enzymes. Technologies were worked out for immobilizing free enzymes or enzyme-producing microbial cells. Improved methods are described for isolation and purification of enzymes.. The bacterial and fungal proteinases are dealt with in more than 10 papers. Similarly, many studies are devoted to the microbial enzymes catalyzing the hydrolysis of starch and cellulose, respectively. The other enzymes studied comprise: ribonucleases, tyrosyl-tRNA-synthetase, ATPase, acid phosphatase, glucose isomerase, alcohol oxidase, lactase, lipase, inulinase, catalase, glucose oxidase, L-aspartase. Two papers deal with the isolation and screening of lignoclastic basidiomycete strains.

Production of microbial exopolysaccharides. The production of alginate *(Azotobacter chroococcum)*, pullulan and xanthan was studied.

Single cell proteins (SCP). Production, chemical analysis and biological testing of SCP produced by methylotrophic bacteria were the themes of 9 papers. Production of SCP by means of a *Hansenula anomala* strain cultured on a mineral medium with ethanol addition was also investigated.

Microbial vitamin synthesis. A *Torulopsis candida* strain was found to be a good riboflavin producer.

Production of ergotic alkaloids. Seven papers deal with various aspects of the production of ergotic alkaloids by *Claviceps purpurea* and *Cl. paspali.*

Production of antibiotics. Biosynthesis of bacitracin, streptomycin, tetracycline, oxytetracycline, nistatin and/or metabolism and ultrastructure of their producers are the topics of 8 papers. The antimicrobial activity of a glycoalkaloid extracted from tomato plants was also investigated.

Food and fodder microbiology. The following problems were studied: obtaining of inverted sugar by using a fungal invertase; antibiotics in milk; toxigenic moulds in some barley and malt samples; technology for obtaining high fructose corn syrup.

Ethanol production. A procedure was elaborated for ethanol production with immobilized viable yeast cells. Another paper describes an optimized technology for ethanol production from sugar beets. Other practical aspects of the alcoholic fermentation were also dealt with (removal of bitter substances from the sedimented beer yeast biomass; reutilization of the residual wine yeast biomass; microbiological efficiency of filtering wine through some indigenous cardboard filters).

Genetic engineering. This part of the volume consists of two review: „Genetic manipulations and their implications in biotechnology" (D. Moldoveanu, I. Vătafu and N. D. Topală); „Fusion of bacterial protoplasts and genetic analysis of recombinants" (E. Săsărman, C. Sârbu, E. Mocanu and. D. Dobrovolski) and 8 original papers having the following topics: isolation and fusion of protoplasts of some industrial yeast strains; fusion of corynebacterial protoplasts; *in vivo* cloning of α-amylase genes from *Bacillus licheniformis* and *B. subtilis;* isolation and purification of some small plasmids from *E. coli* and *B. subtilis;* induction of *B. subtilis* mutants defective in producing α-amylase; a rapid method for obtaining phage DNA; transfer of the tryptophan operon of *B. subtilis;* obtaining of L-tryptophan-producing diauxotrophic mutants of *Corynebacterium glutamicum.*

Mathematical modelling. A mathematical model was developed for aerobic growth of *Saccharomyces cerevisiae* cells.

Culture collections of industrial microorganisms. Two papers are devoted to this topic.

Microbial enhanced oil recovery (MEOR). MEOR is dealt with in 6 papers, two of which review the results of MEOR field trials carried out both in Romania and abroad (I. Lazăr and P. Constantinescu) and the studies of microorganisms producing biopolymers and biosurfactants to be used in in situ enhanced oil recovery processes (I. Lazăr, A. Grigoriu, V. Velehorschi and. S. Dobrotă). Three original papers describe microbiological experiments and the fourth original paper presents the physico-chemical characteristics of the fluid extracted from petroleum reservoirs previously submitted to microbiological treatment.

Microbial leaching of low-grade ores. This topic is reviewed by F. Săftoiu, M. Toniuc and I. Lazăr, then two papers describe the results of leaching low-grade Mn, Pb and Zn ores or poor concentrates by using thiobacilli or fungi (*Penicillium* and *Aspergillus* strains).

Biodeterioration. The studies concern the microorganisms developing in the water used for recovery of ethylene oxide, on walls of different buildings, on coatings of synthetic fibres and during pulp and paper processing. The chemical control of these microorganisms was also studied. Two papers deal with the microbial degradation of xanthan and lignin, respectively.

Bioreactors. Design, manufacture, testing and using of the BIOFOR reactors are described. The recovery of biological heat from a biosynthesis plant was achieved by using an absorption heat pump. In another paper, also concerning bioreactors, the effect of static mixers on oxygen mass transfer was investigated.

Biotechnology of plant cells. Two reviews deal with this problem: „Use of the principles of industrial microbiology in the biotechnology of plant cell" (B. Tesio, D. Nicolescu, D. Iacob and E. Nichiforescu); „*In vitro* cultivation of plants. A new biotechnology" (D. Cachiță-Cosma and V. Cristea).

Biofuel cells. A new biofuel cell was developed. The suspension of two bacteria (*Clostridium butyricum* and *Staphylococcus aureus* Oxford) was used to convert the chemical energy of substrate into electrical output.

Methanogenesis. In a study the autotrophic methanogenic bacteria were used as free or immobilized cells for producing methane from CO_2 and H_2. Other studies have proved that some associations of methanogenic bacteria are able to produce methane from CO_2 when cultured on media prepared from mineral waters, oil brines or sea water.

Waste water treatment. The problem of biodegradability was studied in the case of many chemical pollutants present in different industrial waste waters. The microbial degradability of 2-ethylhexanoic acid and trimethylol propane, alkyl amines and alkylene amines, caprolactam and terephthaloyl chloride, *p*- and *m*-phenylene diamine and phenol was demonstrated.

Use of biological sludges as fertilizers. The effect of unfermented, fermented fluid and fermented dewatered sludges from the petrochemical industry on some soil microbiological and biochemical parameters was studied in a 2-year field experiment. The effect was negative in the first year and positive in the second one.

The researches described in this volume were performed under laboratory, pilot plant and industrial production conditions. Their results are valuable from both fundamental and practical viewpoints.

The volume as a whole reflects and will stimulate the continuous development of industrial microbiology and biotechnology in our country.

ȘTEFAN KISS

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Revista științifică a Universității din Cluj-Napoca, **STUDIA UNIVERSITATIS BABEȘ-BOLYAI,** apare începînd cu anul 1986 în următoarele condiții:

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