ACTA Biologica

ACADEMIAE SCIENTIARUM HUNGARICAE

TOMUS XXIV
FASCICULI 1–2

AKADÉMIAI KIADÓ
BUDAPEST 1973

ABAHAU 24(1–2) 1–130 (1973)

INDEX

ACTA BIOLOGICA

A MAGYAR TUDOMÁNYOS AKADÉMIA BIOLÓGIAI KÖZLEMÉNYEI

SZERKESZTŐSÉG: 1082 BUDAPEST VIII., ÜLLŐI ÚT 78. KIADÓHIVATAL: 1363 BUDAPEST, ALKOTMÁNY U. 21.

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TOMUS XXIV

AKADÉMIAI KIADÓ, BUDAPEST

1973

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INDUCED PRODUCTION OF BETA-GALACTOSIDASE IN STREPTOMYCES GRISEUS

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(Received 1972—01—29)

Abstract

Authors investigated the induced production of beta-galactosidase in *Streptomyces griseus*. They determined the optimal circumstances of induction, then established that the maximum production of the enzyme needs 2×10^{-3} mol/l lactose. The application of the inducer is followed by a lag phase of 1—1.5 h, then the enzyme level increases, reaching a maximum, 4.5—6 times the basal level after about 5 h. Melibiose, galactose and isopropyl-beta-D-thiogalactopyranoside displayed no inductive effect. The induction could be inhibited by actinomycin D, namely 1 μg/ml causes 40% and 10 μg/ml a total inhibition, if administered together with the inducer.

Introduction

Investigations into the inductive enzyme production of living organisms are used for studying the control of protein synthesis. The best-known regulation mechanism is that of the lac operon of *E. coli*. Investigating this system, JACOB and MONOD [5] published their theory concerning the regulation of bacterial protein-synthesizing mechanism. Inductive beta-galactosidase production has also been described in other organisms, namely, in Gram negative [1, 13], Gram positive [3, 6, 11] bacteria and eucellulae [2, 10]. This enabled a comparison of several protocellular and eucellular aspects of regulation of beta-galactosidase production.

In the present paper the inductive beta-galactosidase production of *Streptomyces griseus* is analyzed. This species represents a higher level of organization within the protocellulae than does the *E. coli*, therefore, it presumably displays a more differentiated regulation system. Hence, the investigation of the inductive beta-galactosidase system of this species and its comparison with those of organisms showing a different level of organization seem to be of interest.

Material and method

The variant No. 52-1(301)A of *Streptomyces griseus* producing streptomycin was used throughout.

Germination was carried out in 500 ml Erlenmeyer flasks, using 200 million spores per 100 ml germinating medium, at 27 °C, in shaken liquid culture. The germinating medium ("synthetic medium F") was composed as follows; 0.2 g NH_4NO_3; 0.12 g KH_2PO_4; 0.29 g

K_2HPO_4; 0.4 g $CaCO_3$; 0.1 g NaCl; 0.1 g $MgSO_4$; 0.00064 g $CuSO_4 \cdot 5\ H_2O$; 0.0001 g $FeSO_4 \cdot 7\ H_2O$; 0.00015 g $MnCl_2 \cdot 4H_2O$; 0.0002g $ZnSO_4$; 0.004g sodium hexametaphosphate per 100 ml distilled water. Two per cent starch and 0.5% Proteose peptone were added to this medium, and the pH was adjusted to 7.4. The germination took 22 h.

Induction. The germinated, 22-h-old culture was centrifuged, then washed once in a 1/15 mol/l phosphate buffer of pH 7.0, and resuspended in the original volume (100 ml) of the synthetic medium F containing 0.5 % tryptone. The inducer was added to this basic medium and so were the substances described in each experiment. The further culturing took place in glasses of 20×60 mm size (penicillin ampoules) containing 3 ml culture in a shaker at 27 °C. The washing medium used for the investigations on substrate stabilization was composed of 500 μg/ml chloramphenicol in synthetic medium F containing 0.5% tryptone and 2% lactose or glucose.

Determination of beta-galactosidase and total protein. At each sampling the contents of 2 ampoules were analyzed, considered as parallels, and the determinations of beta-galactosidase and total protein were carried out separately in each parallel. Before the determinations, the cultures were washed 3 times with 1/15 mol/l phosphate buffer of pH 7.0 at 3 °C, then resuspended in the same buffer to the original volume (3 ml), and treated in ultrasonic desintegrator (1.5 A, 1.5 min). The determination of beta-galactosidase was performed according to LEDERBERG [7] as follows: 1 ml sonicated mycelia with 1.5 ml *o*-nitrophenyl-beta-D-galactopyranoside (ONPG) of 1 mg/ml concentration was incubated at 37 °C for 20 min, then the reaction was stopped with 1 ml 0.1 mol/l Na_2CO_3. After this, the mixture was centrifuged and the absorption of the supernatant at 420 nm was measured in a Unicam SP 1 800 spectrophotometer. The protein content was measured in the sonicated mycelia according to LOWRY [9] compared to a standard bovine albumin solution.

Results

The optimal circumstances of the induction. Cultures germinated for 22 h were used in the experiments. *Streptomyces griseus* grows very intensely in the medium used for germination, however, it produces only little beta-galactosidase. The optimal conditions of induction were selected by investigating the following six parallel cultures:

Culture 1. The mycelia were cultured further in the unchanged germinating medium.

Culture 2. To the germinated culture 4×10^{-3} mol/l lactose was added and unchanged circumstances were maintained.

Cultures 3, 4, 5 and 6. The 22-h-old culture was washed twice in 1/15 mol/l phosphate buffer of pH 7.0, then resuspended in the original volume of synthetic medium F. After dividing the culture into 4 parts, the synthetic medium F was completed as follows (final concentrations):

Culture 3: 4×10^{-3} mol/l lactose;
Culture 4: 4×10^{-3} mol/l lactose and 0.5% tryptone;
Culture 5: 2% glycerol;
Culture 6: 2% glycerol and 0.5% tryptone.

The centrifugation, washing and resuspension were performed in about 40 min. The sampling was carried out 1, 3, 7 and 21 h, after restarting of incubation. The beta-galactosidase activity and total protein concentration were determined in each sample.

The results are summarized in Fig. 1. It shows no essential difference between the total protein concentrations measured in the cultures 1—6. The slightly higher values for cultures 4 and 6 as compared with those for cultures 3 and 5 can probably be explained by the better conditions of growth in the presence of tryptone.

The beta-galactosidase activity was apparently much higher in the cultures containing lactose. The highest induction rate was achieved if the mycelia

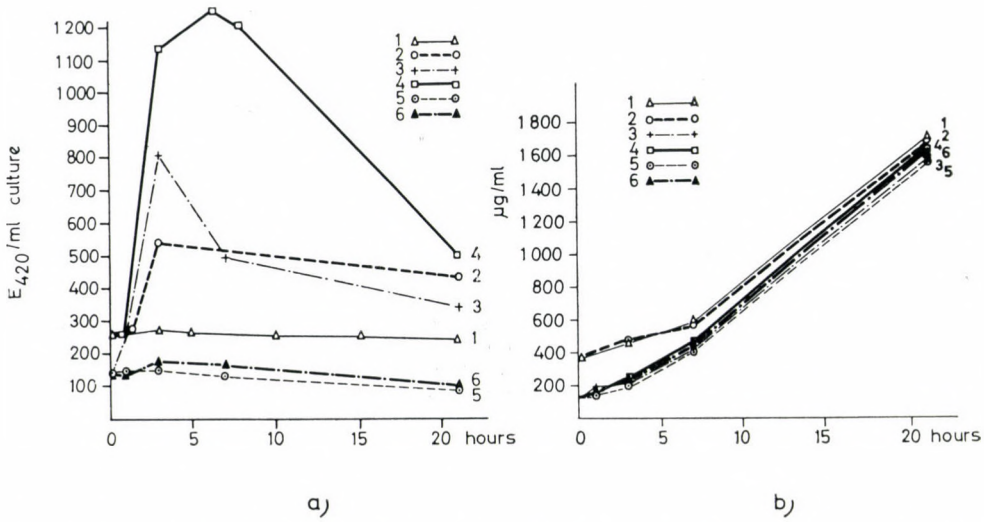

Fig. 1. Determination of the optimal conditions of induction. a=Beta-galactosidase activity; b=total protein. Numbers 1—6 represent cultures 1—6 (see text). 0 h represents the restarting of cultivation

had been washed out from the germinating medium and the fresh medium contained an inductive concentration of lactose together with 0.5% tryptone.

The effects of different carbohydrates and isopropyl-beta-D-thiogalactopyranoside (IPTG). Apart from lactose, the inductive effects of melibiose, galactose and IPTG were investigated. Fig. 2 shows that the growth of different cultures was nearly parallel, whereas the beta-galactosidase activity was higher than that of the control only in the presence of 4×10^{-3} mol/l lactose. Melibiose, galactose and IPTG failed to induce the synthesis of beta-galactosidase in *Streptomyces griseus*.

Determination of the lactose concentration exerting the highest inductive effect. According to Fig. 3, the increase of the total protein in control and experimental cultures containing different concentrations of lactose, is identical. 10^{-3} mol/l lactose is sufficient to induce a 3.7-fold increase in the specific activity. The inductive effects of 2×10^{-3} and 4×10^{-3} mol/l lactose concentra-

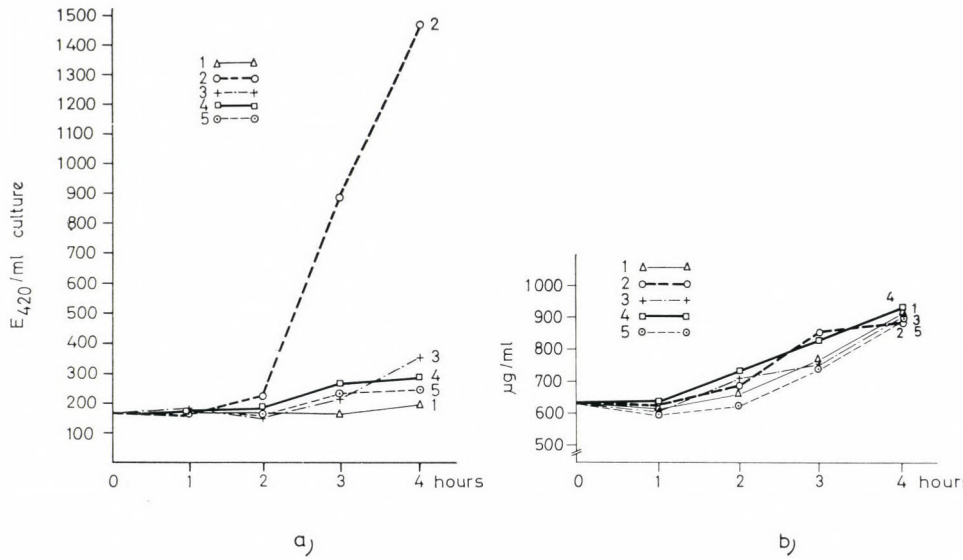

Fig. 2. Effect of lactose, melibiose, galactose and IPTG on the beta-galactosidase production of *Streptomyces griseus*. a=Beta-galactosidase activity; b=total protein content. Mycelia were washed after a germination of 22 h, then cultured in, 1 = the basic medium; 2 = in the presence of 4×10^{-3} mol/l lactose; 3= in the presence of 4×10^{-3} mol/l melibiose; 4 = in the presence of 4×10^{-3} mol/l galactose; 5=in the presence of 10^{-3} mol/l IPTG

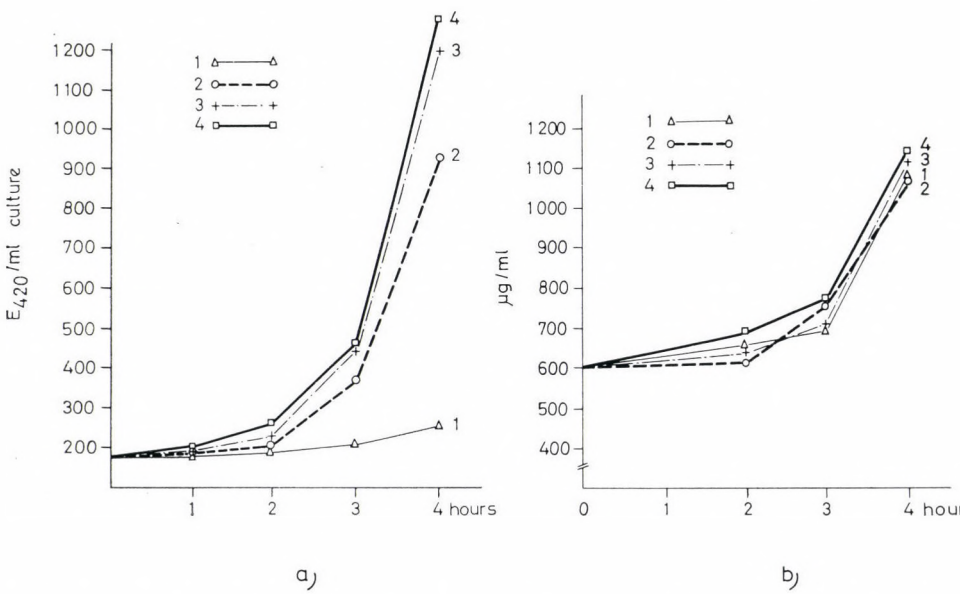

Fig. 3. Determination of the lactose concentration of optimal inductive effect. a = Beta-galactosidase activity; b = total protein. Mycelia were washed after a germination of 22 h, then restarted for cultivation at 0 h, 1 = in the basic medium; 2 = in the presence of 10^{-3} mol/l lactose; 3 = 2 × 10^{-3} mol/l lactose; 4 = 4×10^{-3} mol/l lactose

tions can be regarded as equivalent, the increase in specific activity being nearly five-fold in both cases. No higher increase in specific activity was achieved using even higher concentrations of lactose. Thus, 2×10^{-3} mol/l lactose showed a maximal induction of beta-galactosidase.

The time-dependence of the induction. The Figs 1 and 4 demonstrate that beta-galactosidase activity started to increase intensely 1—1.5 h after the

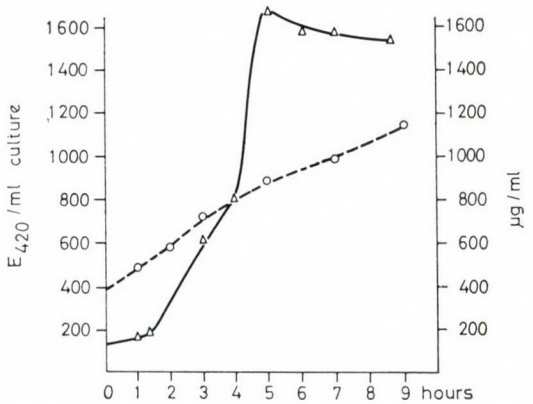

Fig. 4. The time-dependence of induction. Incubation took place as described in the footnote to Fig. 3 with 4×10^{-3} mol/l lactose administered at 0 h. Continuous line = beta-galactosidase activity; dotted line = total protein

induction, it reached a maximum after 5 h, then slowly decreased. The total protein increased evenly.

Investigation on the substrate stabilization. The increase of beta-galactosidase activity in the presence of lactose indicates an induction phenomenon. However, in order to support the hypothesis of induction, it is necessary to exclude the possibility of a stabilizing effect of substrate maintained by lactose, the specific substrate of beta-galactosidase. Four hours subsequent to the addition of lactose, the further protein synthesis was blocked in the cultures by adding 500 µg/ml chloramphenicol. The culture was then divided into two halves and washed 3 times with the washing medium, then resuspended in washing medium containing either lactose or glucose and cultured further, meanwhile the decomposition of beta-galactosidase was investigated. According to Fig. 5, in the presence of lactose or glucose the rate of enzyme decomposition can be regarded as identical, therefore the phenomenon of substrate stabilization can be excluded. Fig. 5 also shows the decomposition of the total protein which like that of the beta-galactosidase is of biphasic pattern.

Effect of actinomycin D on the induction. Lactose induces *de novo* synthesis of beta-galactosidase as suggested by the induction-inhibiting effect of

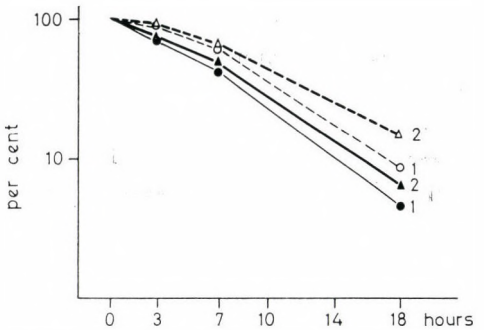

Fig. 5. Inactivation of beta-galactosidase (continuous lines) and total protein (dotted lines). After a germination of 22 h the mycelia were washed, then incubated for 4 h in the presence of 4×10^{-3} mol/l lactose. At this time, 500 μg/ml chloramphenicol was added. The culture was divided into two halves, washed with media containing either lactose or glucose, then cultured again from 0 h, in the presence of 1 = 2% lactose and 2 = 2% glucose. Both cultures contained 0.5 % tryptone and 500 μg/ml chloramphenicol in the synthetic medium F

actinomycin D (Fig. 6). The concentration-dependent effect of 5 μg/ml or more actinomycin D is shown in Fig. 7. Analyzing the effects of 0.25, 0.5 and 1 μg/ml concentrations of actinomycin D, one can establish that the inductive beta-galactosidase production was maintained at these concentrations. In the presence of the two lowest concentrations of actinomycin D, the enzyme production was inhibited to a very low extent as compared to that of the induced, actinomycin D-free cultures. A considerable inhibiting effect was shown by 1 μg/ml, namely, the enzyme level was only 40% of the control value after 5 h. At the same time, the total protein concentration amounted to 75% of that of the control cultures.

Discussion

The induced production of beta-galactosidase was investigated in *Streptomyces griseus*. Comparing this production with that of other protocellular or eucellular organisms as well as with the induced production of mannosidase [4] shown by the same species, the following conclusions can be drawn.

In case of *E. coli* the basal level of the inducable enzyme is very low, it produces practically no beta-galactosidase without inducer. According to our results, the basal level of this enzyme is relatively high in the species studied and during the period of investigation this level proved to be constant, which, however, is low as compared to the level of induction.

A similar phenomenon has been described in connection with the inductive mannosidase of *Streptomyces* [4], therefore, the authors regard as more

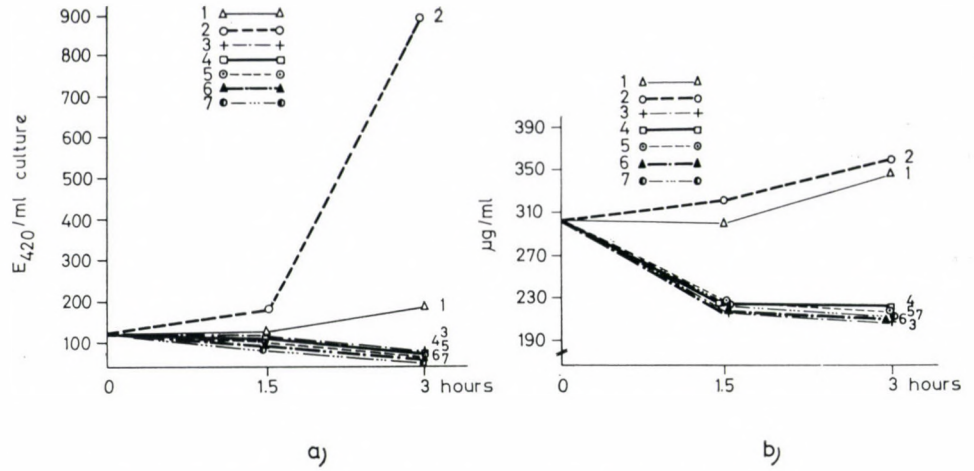

Fig. 6. Effect of actinomycin D on the induced production of beta-galactosidase. a = Beta-galactosidase activity; b = total protein. After a germination of 22 h, the mycelia were washed, then incubated in the basic medium (curve 1), in the presence of 4×10^{-3} mol/l lactose (curves 2—7). From curve 3 to curve 7 increasing amount of actinomycin D was added, 5, 10, 20, 50 and 100 µg/ml, respectively. Cultivation was restarted at 0 h

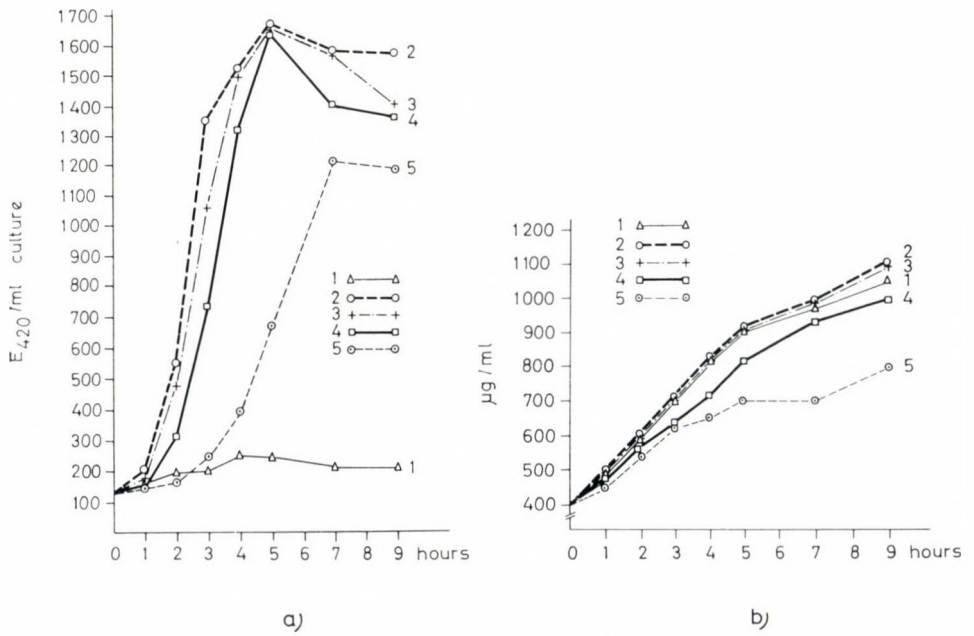

Fig. 7. Effect of actinomycin D on the induced production of beta-galactosidase. a = Beta-galactosidase activity; b = total protein. The experiments were performed as described in Fig. 6. Curve 1 = incubation in basic medium; curves 2—5 = in the presence of 4×10^{-3} mol/l lactose. From curves 3—5 = in the presence of 0.25, 0.5 and 1.0 µg/ml actinomycin D, respectively 0 h = restarting of cultivation

correct to consider the enzyme not to be inductive but semiconstitutive. Similarly, higher levels of basal activities have been mentioned in eucellular systems [2, 10].

Significant differences appeared also in the order of magnitude of maximal enzyme levels observed during induction. Whereas in protocellulae a 1 000 to 10 000-fold increase was observed as related to the basal level, in *Streptomyces griseus* the increase was as low as 4.5—6-fold. The inductive increase rate of the beta-galactosidase level observed in *Neurospora crassa* was of the same order of magnitude (tenfold) [2].

In the protocellular systems the inductive effect can be measured within several minutes (3 min) following the administration of the inductive substance. In the case examined by us an increase of the enzyme level became measurable as late as after 1—1.5 h. As regards the mannosidase production, an even longer (6 h) lag phase has been reported, and the inductive beta-galactosidase production of *Neurospora* became measurable 2 h subsequent to the induction [2, 4].

The causes of the long lag phase measured in our system have not been clarified. However, we established that the lag phase was equally 1—1 h when using either 2×10^{-3} mol/l lactose, or lower effective concentrations.

In *Streptomyces griseus* the initial long lag phase was followed by an increase in the enzyme level with a maximum reached by the 5th hour. This was followed by a decrease, much slower than that of the rate of increase was and the enzyme level did not reach the control value even after 21 h. Meanwhile the total protein content steadily increased. The maximum enzyme level was measured after 24 h in case of induced mannosidase production in *Streptomyces*; in eucellular systems the maximum was reached at 40 h [2].

In *E. coli* the increase of the enzyme level was linear in the logarithmic phase, if the concentration of the inducer was constant [5]. In *Streptomyces griseus* the increase was not linear. It should be considered when interpreting the results that the beta-galactosidase production of *E. coli* can be induced by non-metabolized, artificial inducers [IPTG, methyl-beta-thiogalactopyranoside (TMG)], thus the constant level of inducer concentration can be maintained. The lactose proved to be inductive in our experiments, however, it is metabolized by *Streptomyces griseus*. Therefore, the lactose concentration depends on the actual metabolic activity of the cells. It should also be considered that the *E. coli* culture in the logarithmic phase can be regarded as a homogeneous, more or less synchronized system. In case of *Streptomyces griseus* the mycelia of the same culture contain both morphologically and functionally different hyphae [15, 16].

As regards the specificity of the inducer, the different beta-galactosidase systems display very divergent behaviour. In the case of *Streptomyces griseus*, only lactose proved to be of inductive character, whereas melibiose, galactose

and IPTG did not. A beta-galactosidase induceable only by lactose has been described by ANDERSON and RICKENBERG [1].

A maximum inductive effect was evolved by 2×10^{-3} mol/l lactose in *Streptomyces griseus*. As regards the order of magnitude of this concentration, this value lies nearer to the inducer concentrations (10^{-3}) necessary for other protocellular systems than to those (10^{-2}) required by eucellular ones [2, 10].

Unlike *E. coli* [14], *Streptomyces griseus* is permeable for actinomycin D. The beta-galactosidase system of the latter is sensitive to this drug; 1 μg/ml actinomycin D depresses the beta-galactosidase production to a level of 40%, whereas a concentration of 10 μg/ml results in a complete inhibition of induction. As regards the sensitivity to actinomycin D, the induced beta-galactosidase production by *Streptomyces griseus* is less sensitive than e.g. the induced penicillinase production by *Bacillus subtilis* [12], however, it is of similar sensitivity as the induced beta-galactosidase production by *E. coli*, and the induced mannosidase production by *Streptomyces griseus* [4, 8].

Acknowledgements

Authors express their thanks to S. VITÁLIS for the isolation of *Streptomyces griseus* producing inductive beta-galactosidase, to Miss I. SZOBOSZLAI for her technical help and to Mr. F. FÁBIÁN for drawing the figures. Authors are also grateful to Miss Zs. MAGYAR for the personal communication regarding the *Streptomyces griseus* cultures producing beta-galactosidase.

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Acta biol. Acad. Sci. hung., **24**(1—2), 11—17 (1973)

ALTERATION OF RNA SYNTHESIS IN VITRO WITH AN ENDOGENOUS REGULATING FACTOR OF CYTODIFFERENTIATION OF STREPTOMYCES GRISEUS

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(Received 1972—05—18)

Abstract

Effect of Factor C, a previously isolated, non-toxic, heat-labile initiating factor of cytodifferentiation produced by *Streptomyces griseus*, has been examined in different RNA-synthesizing systems *in vitro*. It inhibits both the endogenous synthesizing activity of native DNP complex from *S. griseus* and the binding of purified *E. coli* RNA polymerase to the complex. At higher ionic strength, it also shows a stimulating effect on the exogenous polymerase. The inhibitory effect of Factor C on RNA polymerase can also be observed with purified DNA as template. All the effects of Factor C on native DNP complex are species-specific. They are observable on aggregate of *S. griseus* only; it is ineffective in similar preparations obtained from *E. coli* K 12. These effects of Factor C on RNA synthesis can be in correlation with its activity on the cytodifferentiation of the *S. griseus*.

Introduction

Factor C is a non-toxic endogenous substance which can initiate the conidium-forming differentiation process of the non-sporulating 52-1 strain of *Streptomyces griseus* [9]. Its mode of action has been studied in different systems [5, 7]. It has been assumed that at least some of its components can bind to DNA and its effect on the cytodifferentiation may originate from an alteration of the genic transcription process.

In the present experiments the effect of Factor C on RNA synthesis *in vitro* was examined under different conditions.

Material and method

Strains and cultivation. Streptomyces griseus strain *No. 52—1* was grown on either DIFCO broth medium (beef extract, 0.3%; DIFCO bactopeptone 0.5%; NaCl, 0.5%; glucose, 0.1%; 0.005 mol/l tris-malate buffer pH 7.6) for 24 h or in soy bean — corn steep medium for 72 h [6]. *Escherichia coli* K 12 was grown on meat extract — peptone medium as described earlier [8] and harvested in the log phase. *Streptomyces griseus* 45-H was grown on soy bean—corn steep medium.

Isolation of Factor C. Cell-free fermentation liquid of 72-h culture of *S. griseus* 45-H strain was adjusted to pH 2.4 with sulphuric acid and the precipitate removed by centrifugation. The supernatant was adjusted to pH 3.4, saturated with ammonium sulphate and left

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overnight in the cold. The precipitate was dissolved in distilled water, its pH was adjusted with NaOH to 8 and dialyzed against the same for 24 h. The dialysate was lyophilized. Portions of this powder were dissolved in distilled water, adjusted to pH 8 and were put on Sephadex G-75 column equilibrated with distilled water and the activity was obtained in the exclusion volume. This fraction is about 70 times more active than the original fermentation liquid in cytomorphological test.

RNA polymerase was isolated from *E. coli* K 12 according to a modified method of the Chamberlin and Berg's procedure [1]. The specific activity of the polymerase preparation, immediately after its isolation, was 318 U/mg protein with calf thymus DNA as template.

Isolation of native DNP complexes has been described elsewhere [8]. Briefly, the washed cells were suspended in TMA medium (0.01 mol/l tris-HCl pH 7.8; 0.01 mol/l magnesium acetate; 0.06 mol/l KCl; 0.006 mol/l mercaptoethanol), disrupted in Braun mechanical shaker, the homogenate centrifuged at 6 000 *g* for 30 min, 105 000 *g* for 1 h, then at 150 000 *g* for 4 h. The final pellet was homogenized in a Potter-Elvehjem homogenizer and washed once in 10 vol of TMA medium under the same conditions.

RNA polymerase assay was the same as described earlier [8] except that 100 nmol of 2-^{14}C UTP (1μC/μmol) was added to the samples as label. All the components of the system were mixed together in the cold, including Factor C when present. Systems containing highly-polymerized DNA (BDH, England) as template were started by the addition of the polymerase while the samples containing native DNP complexes were started by the addition of the latter. The RNA synthesis was measured at two different ionic strengths. Lower ionic strength means in 0.25 ml: 1 μmol of magnesium acetate; 6 μmol of KCl. Higher ionic srength means 7.5 μmol of magnesium acetate and 32.5 μmol of NH_4Cl.

All the experiments were repeated several times on different preparations and the results of typical experiments are presented. For all other methodical details see [8].

Results

Effect of Factor C on endogenous RNA-synthesizing activity of native DNP complexes

The DNP complexes, called also aggregates, are native washed nucleoprotein preparations pelleted from homogenates of *S. griseus* or *E. coli*. They are regarded to be structures retaining at least part of the regulating compounds found on the surface of DNA. They show endogenous RNA polymerase activity in the presence of the four nucleotide triphosphates. Factor C inhibits the endogenous RNA polymerase activity of DNP from *S. griseus* while it is ineffective on aggregate from *E. coli* both at lower and higher ionic concentrations (Table 1).

Effect of Factor C on template activity of native DNP complexes

Increasing amounts of aggregate were added to systems containing the same quantity of *E. coli* RNA polymerase. The measurements were carried out both at low and high ionic strength (Fig. 1). RNA polymerase activity increases in parallel with increasing amounts of aggregates to a certain point where curves level off. This point shows the saturating amount of the template for the enzyme present. With excess template amounts, the activity of the RNA polymerase is higher at higher ionic concentrations.

Table 1

Effect of factor C on endogenous RNA polymerase activity of aggregates from E. coli and S. griseus

Aggregate	Ionic strength	UMP incorporated pmol/min/10 μg DNA
E. coli	low	12.92
E. coli + Factor C	low	13.03
E. coli	high	19.52
E. coli + Factor C	high	18.20
S. griseus	low	1.13
S. griseus + Factor C	low	0.76
S. griseus	high	4.03
S. griseus + Factor C	high	3.16

The *S. griseus* aggregate originates from 24-h DIFCO broth culture. The samples contain aggregate of 23 μg of DNA. The *E. coli* aggregate was isolated from log-phase culture and aliquots of 30 μg of DNA were added to the polymerase system. Incubation time was 30 min and 30 μg of Factor C was added as indicated. The figures are the means for duplicate samples

On control curves (without addition of Factor C, continuous lines in Fig. 1 A) of *E. coli* aggregate it is seen that aggregate corresponding to 5 μg of DNA can bind all the enzyme of 35 μg protein. At high ionic concentration a larger amount of aggregate is necessary to saturate the enzyme. In case of aggregate from 24-h *S. griseus* mycelium (Fig. 1 B) an amount of 4 μg as DNA can bind polymerase of 26 μg of protein. At higher ionic strength, about 10 μg DNA is enough for the enzyme to operate with maximum speed. In the presence of Factor C (dashed lines in Fig. 1) the aggregates of the two microorganisms behaved quite differently. On *E. coli* aggregate the only effect is a slight non-characteristic increase of the template activity at higher ionic strength. On *S. griseus* aggregate (Fig. 1B) Factor C strongly inhibits the binding of the polymerase to the template at low ionic strength. About fourfold amount template is required to bind the same quantity of enzyme as in the control samples. At higher ionic strength the same is observable, even with 27.2 μg DNA the saturation curve does not reach its maximum, i.e. this amount of the template could not bind all the polymerase present. At the same time the activity of the bound enzyme exceeds the plateau value of the control curve. It may be noted that after the effect of Factor C the shape of the template saturation curves of the aggregate from 24-h *S. griseus* mycelium reminds of the curves obtained when using aggregate of 72-h culture of the same strain without Factor C (small diagram inserted in Fig. 1B).

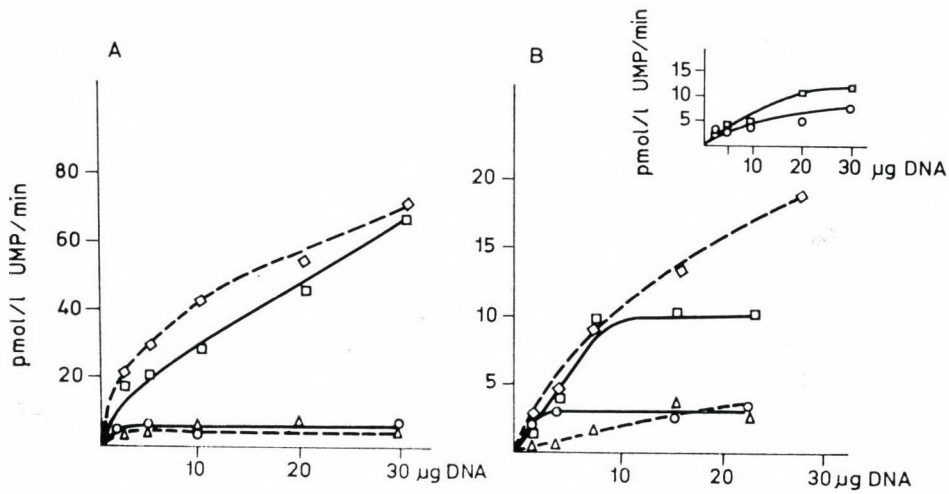

Fig. 1. The effect of Factor C on template activity of aggregates from *S. griseus* and *E. coli*. The incubation time was 30 min. Continuous lines = template saturation curves without addition of Factor C. Dashed lines = the same in the presence of 30 µg of Factor C. ○, △ = lower ionic strength. □, ◊ = higher ionic strength. A = aggregate from log-phase *E. coli* added to 35 µg of polymerase. B = aggregate from 24-h *S. griseus* growing in DIFCO broth. The template was added to 26 µg of *E. coli* RNA polymerase. Inserted diagram in B = template saturation curves of aggregate from 72-h *S. griseus* mycelium growing on soy-bean–corn-steep medium. ○ = lower ionic strength. □ = higher ionic strength. The aggregate was added to 26 µg polymerase. Values of endogenous RNA polymerase activity were always subtracted from the total ct/min values and the differences presented in the diagrams

Effect of Factor C on calf thymus DNA template

Further analysis of the effect of Factor C on RNA synthesis has been made in the presence of purified DNA template. As it is shown of Fig. 2, Factor C inhibits the activity of *E. coli* RNA polymerase added to the system containing calf thymus DNA as template. With increasing concentration of Factor C the inhibition tended to level off at about 50% residual activity. The inhibitory effect could almost be prevented by 10-min heat treatment of Factor C at 100 °C. The incubation of either the DNA template or the RNA polymerase with Factor C before the start of the polymerase reaction resulted in the same degree of inhibition. Factor C was also effective when added to the system 10 min after the start of the polymerase reaction.

Discussion

In earlier studies attempts were made to explore the mode of action of Factor C. It has been established that it increases the actinomycin-D-resistant incorporation of labelled uracil into RNA of several microorganisms, changes the T_m point of purified DNA [7] and interferes with the transient repression

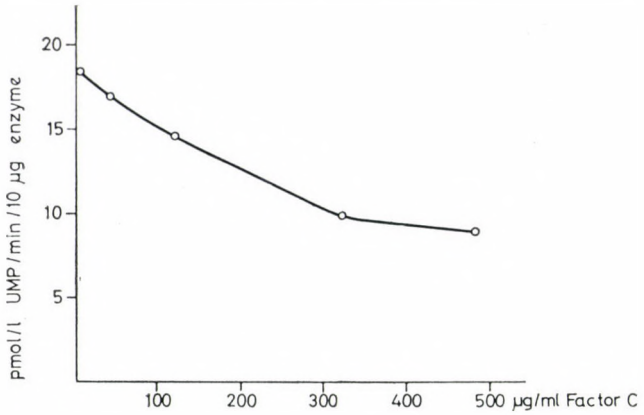

Fig. 2. Effect of Factor C on RNA polymerase activity. The samples contained *E. coli* RNA polymerase of 22 μg of protein, 50 μg of calf thymus DNA and were incubated for 30 min. The experiments were carried out at higher ionic concentration

effect of glucose in β-galactosidase system of *E. coli* [5]. Its effect in suspending the transient glucose repression is rather similar to the effect of cyclic AMP in the same system [3]. These results have rendered it probable that at least some components of Factor C initiating the cytodifferentiation of *S. griseus* have direct effect on the DNA template. By the examination of Factor C in different RNA synthesizing systems *in vitro* we have tried to get more evidence for this direct effect on the genic transcription.

Measuring the template activity both at lower and higher ionic concentrations, two components of the polymerase reaction may be distinguished. It has been described [2] that, at low ionic strength, reinitiation of the RNA chains does not take place. The amount of the template belonging to the saturation point of the curve taken up under these circumstances is inversely proportional to the free sites available on the template for the enzyme to bind. On the other hand, the polymerase activity determined at higher ionic strength is, due to the extensive reinitiation of RNA chains, proportional to the total efficiency of the enzyme on the template.

The effect of heat-labile components of Factor C on template activity of its cognate DNP preparations is complex. At first, Factor C makes a larger part of the binding sites of *S. griseus* aggregate inaccessible for the RNA polymerase. At higher ionic strength Factor C retains this characteristic. The maximal amount of template added cannot exhaust the synthesizing capacity of the polymerase present. On the other hand, with excess template, the polymerase activity exceeds the maximum value obtained without Factor C. From this fact one can assume that although Factor C prevents the synthesis of RNA on some sites of aggregate, the RNA synthesis proceeds with a higher efficiency on uncovered sections. Factor C probably contains several com-

ponents active in alteration of RNA synthesis, some of them having inhibitory effect while others can stimulate the RNA synthesis on the cognate DNP complex at higher ionic strength.

The inhibition of chain initiation probably is not the only site of action. Factor C was effective on the endogenous RNA polymerase activity of *S. griseus* aggregate (Table 1) and it is known that in such aggregates the RNA polymerase activity originates mainly from the elongation of nascent RNA chains that had been initiated *in vivo* before the isolation [4].

Since Factor C was ineffective on aggregate of *E. coli* (Table 1 and Fig. 1), it seems to be species-specific.

Template saturation curves of aggregate of 72-h *S. griseus* mycelium show a decreased polymerase binding capacity in comparison with aggregate from 24-h mycelium. We assume that on the effect of Factor C the template of a young *S. griseus* mycelium gets the property characteristic of the template of a later stage of the life cycle. This can be in correlation with the effect of Factor C on the cytodifferentiation process of *S. griseus*, transforming it from the vegetative into reproductive phase.

Some inhibitory compounds of Factor C are active in polymerase system containing purified DNA template and isolated *E. coli* enzyme. The data presented in the third paragraph of Results support the assumption that it acts *via* binding directly to the DNA template. The existence of residual activity can be explained either by its binding to some defined sites of the template or its competition with the polymerase for the sites of DNA.

On the basis of earlier data we have assumed [8] that in *S. griseus* a more complex regulation system of RNA synthesis exists than in simple bacteria. It is probable that our extract of fermentation liquid called Factor C contains some members of this regulation system. The measurement of RNA synthesis *in vitro* as described here enables us to test the further purification procedure of these components.

Aknowledgement

The authors say many thanks Dr. I. Békési for the Factor C preparations, Mrs. P. Kaszab for technical assistance and Mr. F. Fábián for the documentation.

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Acta biol. Acad. Sci. hung., **24**(1—2), 19—24 (1973)

PHYLOGENESIS OF MAST CELLS

IV. EXPERIMENTAL INVESTIGATIONS ON MAST CELLS OF FISHES

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(Received 1972—05—31)

Abstract

Mast cells of lymphoid type containing alcian blue-positive granules are present in the thymus, the lymph and blood of fish, as well as in the spleen during winter season. In summer, the number of alcian blue-positive mast cells increases in the thymus. The granules of these cells contain serotonin. Endoepithelial formations resembling the calix cells are present in the squamous skin which, in spite of their alcian blue-positive reaction, cannot be classified as mast cells. In contradistinction to other vertebrates, neither the number nor the maturation of mast cells is influenced by cortisone treatment.

Introduction

Numerous literary data indicate the presence of mast cells in all of the vertebrate species [1, 2, 3, 6, 15, 16]. Nevertheless, mast cells seem to differ from each other both morphologically and biochemically. From the biogenic amines, they contain either histamine or serotonin or dopamine [5, 9]. The presence of heparin or some other mucopolysaccharide is obligatory in each mast cell.

Certain cells of fish are considered to be mast cells mainly on the basis of their metachromatic staining, as indicated by the results available for us [13]. Analysing the effect of histamine liberators, VEIL [12] described the presence of large mast cells of intense, metachromatic staining in the squamous skin. According to other investigations [11, 14], a cyclic activity can be observed in the appearance of mast cells.

Our present work was aimed at deciding whether the metachromatic cells of fish suit the requirements of the modern criteria of mast cells (detectability of amines by fluorescence methods, alcian blue staining, etc.). Furthermore, we intended to investigate whether the increased activity of thymus manifesting itself mainly in mast cell production, can be evoked even in fish under experimental conditions. Another question reserving interest is, how far are similar or different the mast cells of fishes as compared to those of other poikilothermic organisms e.g., Amphibia.

Material and method

Fifty adult carps (Cyprinidae) of 600—900 g body weight of both sexes were used. The investigations were carried out during the winter season (from beginning of November till the end of February) as well as during the summer period (from beginning of June till the end of August). During both periods, the animals were divided into an experimental and a control group. The experimental animals were treated with in single dose of cortisone in both periods: they received 15 mg/100 g body weight of cortisone (Adreson-Organon) intramuscularly. Animals were killed 1, 24 and 48 h subsequent the administration of cortisone by decapitation and subsequent mechanical destruction of the spinal cord. The thymus, spleen, blood and lymph (peritoneal fluid) as well as the subsquamous skin surface of the animals were studied. The lymph was obtained by washing the peritoneal cavity by poikilothermic Ringer solution before killing the animal: the fluid obtained was investigated in thick-drop preparations.

The fixation was performed in Carnoy solution. The paraffin-embedded organs were cut in four levels. Thick-drop preparates were made from the blood; the subsquamous skin surface was investigated in both film preparates and sections. The sections were stained with alcian blue—safranin, Azur A (pH 3.0), toluidine blue (pH 6, 7, 9.8), with Giemsa method, and the PAS-reaction was also carried out. Fluorescence was induced in the material fixed in cold absolute ethanol by OPT according JUHLIN and SHELLEY [8], as well as by paraformaldehyde [7].

Results

The winter season

The thymus contains alcian blue-positive mast cells of lymphoid type displaying a well-expressed granulation (Fig. 1) Such cells were found in much smaller numbers in the spleen, where on the other hand Azur-positive mast cells occur in a great number (Fig. 2). There are many Azur-positive cells of mast cell character even in the thymus (Fig. 3), their number outweighs that of the alcian blue-positive ones. PAS-positive cells also occur in the thymus, however, we failed to identify them as mast cells. Alcian blue-positive lymphoid cells can also be found in the lymph and the blood, these cells are also PAS-positive (Fig. 4). Mast cells were not found in the squamous skin. The numerous alcian blue-positive cells of this organ are devoid of mast cell character (Fig. 5). The cytoplasm of these cells is of "foamy" structure, the nucleus is not visible and the cells are much larger than the mast cells occurring in the tissues (Figs 6 and 7). Metachromatic "small" cells also occur here, however, they are alcian blue-negative (Fig. 7).

The large and small metachromatic cells of the squamous skin do not display any fluorescence with the method used. The mast cells of thymus and lymph show a yellow fluorescence characteristic for serotonin. However, this fluorescence is extremely weak, non-convincing.

No difference was observed in the number of mast cells between the control and cortisone-treated animals.

The summer season

The number of histochemically detectable mast cells is very high in the thymus during this season (Fig. 8). The spleen contains the same amount of mast cells as during the winter period. The number of Azur-positive cells is very high in both organs.

The alcian blue-positive cells of the squamous skin are present, their structure is identical with that described in the winter season. The small, alcian blue-negative cells staining metachromatically with toluidine blue, are also present. The most significant difference, as compared to the winter period, is that most of the large cells seem to be emptied (Fig. 9).

The fluorescence investigations revealed serotonin-containing mast cells in the spleen and the thymus.

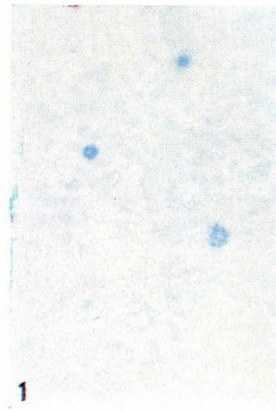
The observations after cortisone treatment were identical with those obtained during the winter season.

Discussion

During our previous investigations on the poikilothermic vertebrate it has been established that the highest degree of mast cell maturation observed in frog (*Rana esculenta*) during winter corresponds to the lowest degree of maturation characteristic for the mammalia [6, 9]. Cortisone treatment supports the maturation of mast cells in the thymus and the picture of mast cells is converted to that corresponding to the summer state [10].

Among the organs examined by us the thymus, the spleen, the lymph and the blood of fishes contained mast cells. They were young, alcian blue-positive mast cells, displaying also serotonin fluorescence. Nevertheless, more matured forms could not be observed even during the summer season. Intense mast cell reaction appeared neither after cortisone treatment, indicating that this hormone does not induce mast cell maturation. On this basis we assume that in fish either the enzymatic apparatus of mast cells is unable to produce amines in the amount necessary for the appearance of the safranin-positive granules (this would be supposed by the weak fluorescence), or the glucocorticoids are not regulators of mast cell formation. In the latter case, one can also assume that cortisone can stimulate the maturation of mast cells only in those animals, e.g. Amphibia, in which such maturation does take place even normally; however, it cannot evoke maturation in animals in which this process is absent under normal circumstances.

The metachromatic cells found in the squamous skin are considered to be mast cells in literature mainly on the basis of their heparin content [13]. On the basis of our investigations, these endoepithelial formations of calix cell character cannot be classified as mast cells, since they are different in size,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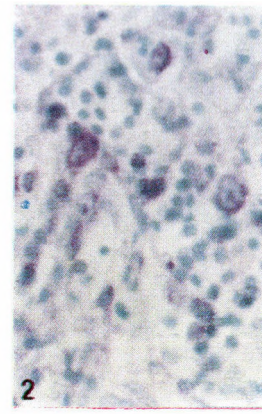
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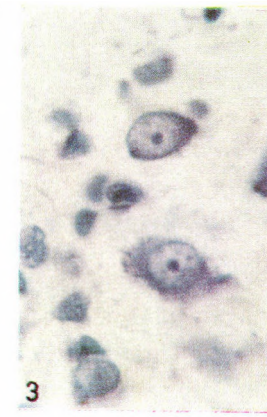
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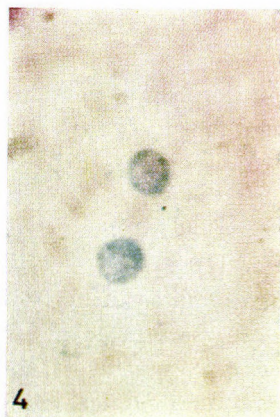
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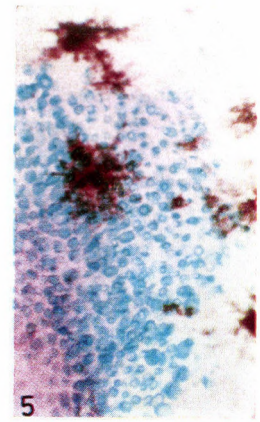
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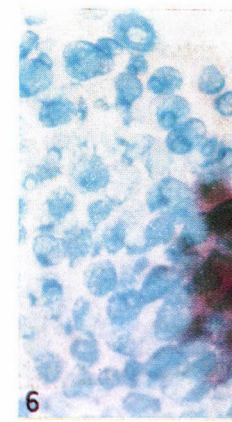
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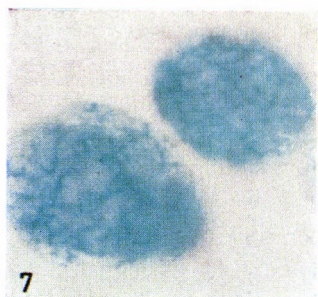
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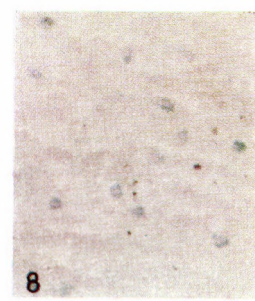
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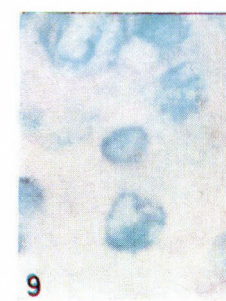
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show no fluorescence and their foamy structure is not characteristic of mast cells. On the other hand, in our present knowledge, the acid mucopolysaccharide content is not sufficient for definition of mast cells [5]. The so-called small mast cells found also in the squamous skin have been interpreted by VEIL [14] as the last stages of degenerating mast cells, since they contain heparin and show toluidine blue metachromasy. The small mast cells proved to be alcian blue-negative in every case during our experiments and displayed no fluorescence. On this basis they cannot be regarded as mast cells. Thus the mast cell seems to be of blood cell character in fish. This is all the more interesting, since the same conclusion was achieved during our previous ontogenetic investigations even in rats [4].

The quantitative differences in the occurrence of Azur A and alcian blue-positive mast cells have drown our attention to the fact that the phenothiazine stain reveals mainly the heparin-containing cells, whereas in the alcian blue-staining, if it is used for mast cells, a significant role is played even by the ratio of heparin to biogenic amines.

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Fig. 1. Alcian blue-positive mast cells in the thymus; winter season. Alcian blue–safranin, ×400
Fig. 2. Azur-positive mast cells in the spleen; winter season. Azur A, ×400
Fig. 3. Azur-positive mast cells of lymphoid character in the thymus; winter season. Azur A, ×400
Fig. 4. Alcian blue-positive mast cells of lymphoid character in the peritoneal fluid (lymph); winter season. Alcian blue–safranin, ×1 000 (oil immersion)
Fig. 5. Alcian blue-positive cells and chromatophores in the subsquamous skin surface; winter season. Alcian blue–safranin, ×100
Fig. 6. Alcian blue-positive cells in the subsquamous skin surface; winter season. Alcian blue–safranin, ×800
Fig. 7. Alcian blue-positive cells in the subsquamous skin surface; winter season. Alcian blue–safranin, ×1 000 (oil immersion)
Fig. 8. Alcian blue-positive mast cells in the thymus; summer season, ×200
Fig. 9. Alcian blue-positive and emptied cells as well as chromatophores in the subsquamous skin; summer season. Alcian blue–safranin, ×320

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Acta biol. Acad. Sci. hung., **24**(1—2), 25—30 (1973)

INSECTICIDES AND SOIL MICROORGANISMS

I. EFFECT OF DIPTEREX ON THE GROWTH OF RHIZOBIUM LEGUMINOSARUM AND RHIZOBIUM TRIFOLII AS INFLUENCED BY TEMPERATURE, pH AND TYPE OF NITROGEN

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(Received 1972—06—05)

Abstract

The optimum temperature for *Rhizobium leguminosarum* and *Rhizobium trifolii* (in culture) was 30 °C. Inclusion of Dipterex in Allison's medium suppressed the growth of both organisms (especially at the two highest concentrations, 3×10^{-3} and 5×10^{-3} mol/ml) and shifted the optimum temperature from 30 to 25 °C. The optimum pH for growth of both rhizobia was 7.0. Inclusion of Dipterex, especially at its two highest concentrations, led to a highly significant decrease in growth and shifted the optimum pH to 6.5. Both organisms are more sensitive to acidity than to alkalinity.

Introduction

Since the discovery that nodule bacteria can enrich the soil, these organisms have become the target of numerous investigations. Experiments have been conducted to study their behaviour and physiology by many workers such as HARRISON [7], VAN SCHREVEN and co-workers [11], NORRIS [9], BURTON and CURLEY [4]. During the last two decades many chemicals have attracted the attention towards their use as insecticides. There is no doubt that the extensive use of these compounds year by year has a profound residual effect on plants as well as microorganisms living in association. In Egypt, the insecticide Dipterex (0,0-dimethyl-2,2,2-trichloro-1-hydroxyethyl phosphonate) has been used in tremendous amounts in controlling cotton leaf worm (*Spodoptera littoralis*), one of the most serious pests affecting the principal economical crop. Since nodulation is a process naturally occurring in soil, the population of the nodule-forming bacteria is probably affected by the accumulated amounts of Dipterex.

The object of the present work was to study the effect of different concentrations of Dipterex on the growth of *Rhizobium leguminosarum* and *Rhizobium trifolii* at various temperature and pH values using different nitrogen sources.

Material and method

Pure cultures of *R. leguminosarum* and *R. trifolii* were isolated on medium "79" (yeast extract—mannitol agar) according to the method described by ALLEN [1], from roots of broad bean (*Vicia faba*) and clover (*Trifolium alexandrinum*). Since the absolute identity of an isolate

as a nodule-forming organism lies in its ability to produce effective nodules on its specific leguminous plants, each of the isolates was tested on its specific host using the technique adopted by ALLEN [1]. Effective nodules (having large size, pink colour and located near the main root) were formed on the roots of the two host plants, indicating that the test was positive.

Temperature experiment. The temperature levels used during this experiment were 15, 20, 25, 30 and 35 °C. For each temperature, 28 Erlenmayer flasks, each of 100 ml capacity, were used. Twenty-five ml of Allison's medium [13] containing 100 parts/10^6 of nitrogen in the form of either $NaNO_3$ or $(NH_4)_2SO_4$ were introduced in each flask. After autoclaving, the flasks were left to cool down before adding Dipterex (10^{-3}, 3×10^{-3} or 5×10^{-3} mol/ml medium). The pH of the medium remained constant during the experiment, due to the presence of the two phosphate salts K_2HPO_4 and KH_2PO_4 in the medium. Inoculation was carried out with one ml of 3-day-old bacterial suspension of either *R. leguminosarum* or *R. trifolii*. After an incubation for 24 hours at its specific temperature, bacterial growth was estimated turbidimetrically as described by BERGERSEN [3].

pH experiment. In the experiment the temperature was kept at 30 °C throughout whereas the pH was varied between 5.5 and 8.5 by adding different amounts of either 0.1 N-NaOH or 1% H_3PO_4 to the medium as recommended by WILSON and KNIGHT [13]. Otherwise the experimental conditions were the same as described above.

Results

Temperature. Table 1 shows the effect of different concentrations of Dipterex on the growth of *Rhizobium leguminosarum* at different temperatures and in presence of $NaNO_3$ or $(NH_4)_2SO_4$. It can be seen that in control samples (where no Dipterex was added) there was a gradual increase in the number of cells with the rise in temperature up to 30 °C where maximum growth was obtained. A remarkable significant decrease in growth occurred at 35 °C, and in preliminary experiments no growth was observed at 40 °C. Below 15 °C the growth was greatly suppressed. Presence of Dipterex at 10^{-3} mol/ml did not affect this behaviour. Higher concentrations, on the other hand, shifted the optimum temperature for growth from 30 to 25 °C. Table 1 also shows that at each temperature level the bacterial yield was the more suppressed the more insecticide was added to the medium. In general, the growth of *R. leguminosarum* was significantly more intensive at the same temperature in the absence than in the presence of Dipterex. Moreover, the maximum growth at 30 °C was significantly higher with $(NH_4)_2SO_4$ than with $NaNO_3$ as nitrogen source a behaviour which indicates a better utilization of the ammonium nitrogen.

The optimum temperature for growth of *Rhizobium trifolii* in the absence of Dipterex was also 30 °C (Table 2). Generally, inclusion of the insecticide in the medium induced a clear significant depression of growth at each temperature; the higher the concentration the more pronounced was the depression. The optimum temperature was shifted from 30 to 25 °C in the presence of insecticide concentrations above 10^{-3} mol/ml. An unexpected increase in growth was obtained at 30 °C when the insecticide was added in a concentration of 10^{-3} mol/ml to the medium containing $(NH_4)_2SO_4$. Moreover, in the presence of Dipterex, regardless of its concentration, the growth was much more intensive with $(NH_4)_2SO_4$ than with $NaNO_3$ at any temperature.

Table 1

Effect of different concentrations of Dipterex on growth vigour of Rhizobium leguminosarum at different temperatures and in presence of either $NaNO_3$ or $(NH_4)_2SO_4$

Temperature °C	Number of cells ($\times 10^7$)/ml of Allison's medium containing							
	$NaNO_3$				$(NH_4)_2SO_4$			
	mol/ml Dipterex							
	Control	10^{-3}	3×10^{-3}	5×10^{-3}	Control	10^{-3}	3×10^{-3}	5×10^{-3}
15	34.0	29.0	29.0	18.0	30.0	32.0	25.0	17.0
20	45.0	33.0	22.5	18.0	40.0	37.0	34.0	20.5
25	59.0	48.0	41.5	27.0	48.0	57.0	31.0	28.5
30	90.0	86.0	30.0	9.0	114.0	90.0	43.0	23.0
35	57.0	23.0	15.0	4.3	58.0	47.0	26.0	12.2

L. S. D. = 5.43×10^7 at 0.05 level and 7.2×10^7 at 0.01 level

Table 2

Effect of different concentrations of Dipterex on the growth of Rhizobium trifolii at different temperatures and in presence of either $NaNO_3$ or $(NH_4)_2SO_4$

Temperature °C	Number of cells ($\times 10^7$)/ml of Allison's medium containing							
	$NaNO_3$				$(NH_4)_2SO_4$			
	mol/ml Dipterex							
	Control	10^{-3}	3×10^{-3}	5×10^{-3}	Control	10^{-3}	3×10^{-3}	5×10^{-3}
15	45.0	32.0	12.5	6.4	43.0	41.0	34.0	25.5
20	60.0	31.0	22.0	3.2	70.0	60.0	55.0	40.0
25	92.0	68.0	32.5	5.7	82.0	68.0	65.0	46.0
30	126.0	85.0	6.0	5.7	86.0	120.0	43.0	17.2
35	110.0	64.0	3.4	5.5	86.0	40.0	17.0	14.3

L. S. D. = 6.65×10^7 at 0.05 level and 8.80×10^7 at 0.01 level

Table 3 shows that in the absence of Dipterex the growth of *R. leguminosarum* was more sensitive to changes in temperature in the medium containing $(NH_4)_2SO_4$ as nitrogen source; unlike this, the growth of *R. trifolii* showed higher sensitivity to temperature if the nitrogen source was $NaNO_3$.

pH. Table 4 shows that the growth of *R. leguminosarum* depends on the pH of the medium, irrespective of the nitrogen source. Thus an increase in pH was accompained by increase in growth up to pH 7.0, beyond which it began to decrease again. The growth of the organism shows a higher sensitivity to acidic rather than to alkaline media. Dipterex, especially its large doses

Table 3

Temperature-dependence of the growth of R. leguminosarum and R. trifolii in the absence of Dipterex with respect to the nitrogen source
Bacterial yield expressed in per cent of the yield at 30 °C

Nitrogen source	Organism	Relation yield at				
		15 °C	20 °C	25 °C	30 °C	35 °C
$NaNO_3$	*R. leguminosarum*	37.8	50.0	65.6	100	63.6
$(NH_4)_2SO_4$		26.3	35.1	42.1	100	50.9
$NaNO_3$	*R. trifolii*	35.7	47.6	73.0	100	87.3
$(NH_4)_2SO_4$		50.0	81.4	95.4	100	100.0

Table 4

Effect of Dipterex on the growth of R. leguminosarum at different pH values in presence of either $NaNO_3$ or $(NH_4)_2SO_4$

pH	Number of cells ($\times 10^7$)/ml of Allison's medium containing							
	$NaNO_3$				$(NH_4)_2SO_4$			
	mol/ml Dipterex							
	Control	10^{-3}	3×10^{-3}	5×10^{-3}	Control	10^{-3}	3×10^{-3}	5×10^{-3}
5.5	21.0	22.0	0.6	0.4	22.0	18.0	14.0	2.0
6.0	75.0	50.0	40.0	20.0	64.0	58.0	39.0	6.6
6.5	80.0	76.0	57.0	44.0	75.0	64.0	62.0	33.0
7.0	89.0	79.0	45.0	16.0	87.0	71.0	55.0	25.0
7.5	70.0	65.0	42.0	19.0	86.0	70.0	59.0	24.0
8.0	69.0	55.0	40.0	9.6	81.0	54.0	44.0	22.0
8.5	70.0	70.0	15.0	5.0	78.0	44.0	40.0	12.0

L.S.D. = 6.02×10^7 at 0.05 level and 7.99×10^7 at 0.01 level

significantly depressed the growth, and the optimum pH was shifted to 6.5. Generally, the presence of nitrogen in the form of ammonium salt appeared to protect *R. leguminosarum* to a significant extent against large doses of Dipterex prevailing at pH values 7.0 and higher.

Data showing the effect of Dipterex on the growth of *R. trifolii* at different pH values in the presence of $NaNO_3$ or $(NH_4)_2SO_4$ are included in Table 5. The growth pattern showed an optimum pH lying at 7.5 with sensitivity tending more towards acidity. At each pH, addition of Dipterex to the culture medium led to a significant depression of growth, which was more pronounced in the presence of the two larger doses. The optimum pH for the organism, too,

Table 5

Effect of Dipterex on the growth of Rhizobium trifolii at different pH values in the presence of either $NaNO_3$ or $(NH_4)_2SO_4$

pH	Number of cells ($\times 10^7$)/ml of Allison's medium containing							
	$NaNO_3$				$(NH_4)_2SO_4$			
	mol/ml Dipterex							
	Control	10^{-3}	3×10^{-3}	5×10^{-3}	Control	10^{-3}	3×10^{-3}	5×10^{-3}
5.5	35.0	25.0	3.5	6.4	30.0	20.0	20.0	8.0
6.0	77.0	50.0	5.7	3.5	76.0	54.0	31.0	13.6
6.5	97.0	75.0	11.0	10.2	70.0	68.0	40.0	30.0
7.0	140.0	82.0	15.0	8.6	90.0	110.0	61.0	35.0
7.5	150.0	66.0	8.6	3.1	103.0	100.0	60.0	30.0
8.0	95.0	63.0	6.0	2.6	90.0	82.0	50.0	28.0
8.5	80.0	80.0	4.0	1.0	88.0	74.0	38.0	20.0

L. S. D. = 5.09 $\times$ 10^7 at 0.05 level and 6.80 $\times$ 10^7 at 0.01 level

was shifted to lower values depending upon the concentration of the insecticide in the medium. A protective effect of ammonium nitrogen against Dipterex toxicity was observed at almost all pH values.

Discussion

The recorded optimum conditions for growth of the two *Rhizobium* spp. under study are in accordance with literary data. Thus ALLISON and MINOR [2] found that the optimum temperature for the growth of *R. leguminosarum*, *R. trifolii*, *R. phaseoli* and *R.* sp. from Dahlea was between 29—31 °C, and no growth occurred at 39 °C. They also stated that the optimum temperature for *R. meliloti* was about 35 °C. According to PEPPLER [10] rhizobia, except *R. meliloti* grow best in the range from 30 to 35 °C. Inclusion of Dipterex in the nutrient medium suppressed the growth of both rhizobia, especially when present in concentrations higher than 10^{-3} mol/ml. The dose-dependence of the suppression was more evident at higher temperatures.

The lower temperature sensitivity of the growth of *R. leguminosarum* with $NaNO_3$ as the only nitrogen source (Table 3) is consistent with BERGERSEN's data. The inverse behaviour in this respect of *R. trifolii* is, however, inconsistent with the findings of the same author. However, MOHAMED [8] found that on adding different substances as the only nitrogen source to Dox's medium, *R. leguminosarum* showed the highest growth on yeast extract followed by autoclaved stable manure, cold-sterilized stable manure, asparagin and ammo-

nium sulphate. The growth was the weakest if $NaNO_3$ was the nitrogen source. This is in agreement with the observed protective effect of $(NH_4)_2SO_4$ against the toxic effect of the insecticide in the present work.

The optimum pH at 7.0 is in agreement with earlier findings by FRED and co-workers [5], WILSON [12] and GRAHAM and PARKER [6]. According to FRED and co-workers [5] all rhizobia are of about the same tolerance to alkaline milieu but their acid-tolerance is variable. The two *Rhizobium* spp. used in the present work were found to be more acid-sensitive. Nevertheless, addition of Dipterex to the culture medium shifted the optimum pH from neutrality towards slight acidity.

It can therefore be concluded that Dipterex exerts a toxic suppressive effect on the growth of both *Rhizobium* spp. at a wide range of temperature and pH. This might, in turn, influence indirectly the process of nodulation in roots of the corresponding hosts. This possibility will be the subject of our forthcoming work.

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Acta biol. Acad. Sci. hung., **24**(1—2), 31—42 (1973)

INTERSPECIFIC CORRELATION OF PLANTS IN OAK-WOOD AT INCREASING BLOCK SIZES

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(Received 1972—06—15)

Abstract

The interspecific correlations of 12 species were analyzed on the basis of their cover values at 7 block sizes increasing from 4 m^2 to 256 m^2. Evaluation was based on the significant interspecific correlations between species-pairs, taking into consideration the sign of correlation. In contiguous populations, correlation may be established through an enforcement or dominance of competition, struggle, compensation, the lack of competition or several other population influences. If two species show unidirectional correlation at some block sizes, their affinity may be attributed to habitat factors. An example for correlation of alternating sign may be when at smaller block sizes struggle and competition (negative correlation), while at larger block sizes parallel to the fading of struggle and competition, positive correlation is prevailing between two species. For positive correlations of the studied species blocks of 16 m^2 area, consisting of 4 basic units, are favoured. The species form a spatial reticulum-like association with each other through their significant correlations. The spatial reticulum is well-developed at small block sizes while it becomes simpler with correlation chains separated, species-pairs losing contact, and several species failing to form significant interspecific correlation at larger block sizes.

Introduction

In the phytocoenological literature, interspecific correlation is studied on the basis of presence–absence values rather than on that of cover values.

The purposes of the present investigations have been to study the number and the sign distribution of significant interspecific correlations (ISC) and to determine the affinity of the species in *Orno-Quercetum* plant community at increasing area sizes (block sizes) taking the cover values of the most frequent species into consideration.

Material and method

Remetehegy hill, located near Budapest, was chosen as the spot for studies on ISC. The description of the sample plot set in a xerothermic calciphilous *Quercus pubescens* forest rich in submediterranean species, the procedure used for mapping as well as the analysis of the species pattern consisting of 19 species were the same as reported previously by Précsényi and co-workers [14]. In a sample plot 32×32 m in size, per cent cover values were estimated for each of the species in contiguous squares of 2×2 m (block size of one basic unit). Thus data for 256 squares served as basic figures. Through a contraction by basic unit pairs of cover values for each species block size of two basic units (= 8 m^2), than block size of 4 basic units

(= 16 m²), etc., up to a block size of 64 basic units (= 256 m²) could be derived. In the previous report mentioned above, an analysis of variance of the same species was made at increasing square sizes according to the method of THOMPSON [15]. Applying this method, it was attained that the, in this case undesirable, "treatment effect" of the heterogeneity of the sample plot, viz. the intergroup SQ-value of the next block size, was substracted from the total SQ = $= \Sigma(x - \bar{x})^2$-values of the existing smaller block sizes. The residual SQ-values thus obtained were considered characteristic of the smaller block size [5].

The correlation coefficients were calculated from the latter values for the 12 species occurring more frequently in the plant community (*Vicia sparsiflora*, *Lithospermum purpureo-coeruleum*, *Oryzopsis virescens*, *Dictamnus albus*, *Geum urbanum*, *Lathyrus niger*, *Polygonatum latifolium*, *Melittis grandiflora*, *Brachypodium pinnatum*, *Polygonatum odoratum*, *Lapsana communis*, *Dactylis glomerata*) since more frequent species were supposed to have best represented the correlations within a plant community.

Results

Since by increasing square size, 7 correlation coefficients can be calculated for each species-pair, a total of 462 correlation coefficients have been obtained for the 66 species-pairs that could be formed from the 12 species. The 103 ISC values significant at the P = 0.05 level serve as the basis for further evaluation. The expression "correlated" used in the following refers to this level of correlation.

The correlations were grouped according to the sign of the correlation coefficient (Tables 1—4).

Table 1

Species-pairs showing positive correlations only

Species-pair	Block size						
Geum—Melittis	1		4		16		
Polygonatum l.—Vicia		2			16	32	
Lapsana—Melittis		2			16		
Vicia—Dictamnus	1		4				
Lathyrus—Melittis		2	4				
Vicia—Polygonatum od.	1					32	
Vicia—Lapsana	1						64
Lathyrus—Oryzopsis	1						
Vicia—Melittis		2					
Polygonatum od.—Melittis		2					
Polygonatum l.—Melittis		2					
Dictamnus—Melittis		2					
Dactylis—Melittis			4				
Lathyrus—Lithospermum				8			
Lapsana—Dactylis				8			

Table 2

Species-pairs showing negative correlations only

Species-pair	Block size						
Oryzopsis–Lapsana	1	2					
Polygonatum l.–Lithospermum	1		4				
Geum–Dictamnus				8	16		
Lathyrus–Lapsana				8		32	
Geum–Brachypodium				8			64
Lathyrus–Dictamnus	1						
Brachypodium–Melittis	1						
Vicia–Oryzopsis	1						
Geum–Oryzopsis				8			
Oryzopsis–Lithospermum					16		
Oryzopsis–Polygonatum od.					16		
Dictamnus–Polygonatum od.					16		
Dictamnus–Lapsana					16		
Geum–Polygonatum l.						32	
Polygonatum l.–Lapsana						32	
Polygonatum od.–Lapsana							64
Geum–Polygonatum od.							64
Brachypodiu m–Lapsana							64

Table 3

Correlations with alternating, initially positive sign

Species-pair	Block size						
Lithospermum–Melittis		2		8	16		64(—)
Lithospermum–Lapsana	1			8(—)	16		
Lithospermum–Dactylis			4	8(—)		32	
Geum–Vicia	1						64(—)
Geum–Lithospermum		2	4(—)				
Lithospermum–Dictamnus		2			16(—)		
Polygonatum od.–Dactylis			4			32(—)	
Oryzopsis–Dictamnus					16		64(—)

Species-pairs showing no correlation at either of the block sizes are the following: *Vicia–Lithospermum*, *Vicia–Dactylis*, *Oryzopsis–Polygonatum l.*, *Dictamnus–Polygonatum l.*, *Dictamnus–Brachypodium*, *Dictamnus–Dactylis*,

Table 4

Correlations with alternating, initially negative sign

Species-pair	Block size						
Oryzopsis–Brachypodium	1(—)	2(—)		8(—)	16(—)	32	
Geum–Lapsana		2(—)	4		16	32	64
Lithospermum–Brachypodium	1(—)	2(—)	4			32(—)	
Lathyrus–Polygonatum l.	1(—)		4	8		32	
Lathyrus–Vicia	1(—)			8	16(—)		
Lathyrus–Dactylis	1(—)	2			16		
Oryzopsis–Dactylis	1(—)	2					
Lithospermum–Polygonatum od.	1(—)				16		
Melittis–Oryzopsis		2(—)	4				
Brachypodium–Polygonatum od.		2(—)					64
Vicia–Brachypodium				8(—)			64
Polygonatum l.–Dactylis				8(—)			64

Table 5

Sign distribution of significant correlations in the blocks

Block	+	−	Total
1	7	12	19
2	12	6	18
4	10	2	12
8	5	9	14
16	9	8	17
32	6	5	11
64	5	7	12
Total	54	49	103

Geum–Lathyrus, Geum–Dactylis, Lathyrus-Brachypodium, Lathyrus–Polygonatum od., Polygonatum l.–Brachypodium, Polygonatum l.–Polygonatum od., Brachypodium–Dactylis.

The signs of the correlations were evaluated not only from the point of view of the species-pairs but also from that of the block sizes (Table 5).

The total number of significant ISCs shown by a species characterizes the affinity of the species (Table 6).

Table 6

Affinity order of the 12 species studied from the species assemblage of Orno-Quercetum forest as shown by the number of significant ISCs

Lithospermum	24	*Geum*	19	*Dactylis*	16
Lapsana	21	*Melittis*	19	*Polygonatum l.*	14
		Vicia	18	*Polygonatum od.*	13
		Oryzopsis	18	*Dictamnus*	12
		Brachypodium	17		
		Lathyrus	17		

Discussion

ISC studies performed in contiguous squares may contribute to the exploration of the structure of plant communities [1] even more so when blocks of increasing size, obtained through the fusion of the mapped areas, are studied [5, 9, 10]. There are several factors that may lead to either the development or the cessation of a correlation between two species or may change the sign of a correlation already existing since all of these factors can exert different effects depending on the block size [6, 7].

The data in the literature reported thus far show that a positive correlation between two species may be the results of some external controlling factor but also of a beneficial impact exerted by one of two species on the other; a negative correlation may develop at that scale where direct but unfavourable effects (either physical or physiological-biochemical) exerted on the partner are still prevailing [see most recently 2, 12, 13, 17, etc.]. The expansion of the individuals (groups of shoots) may be decisive even in cases of correlations calculated on the basis of presence–absence data (cases of spatial exclusion) [6].

There are only few reports estimating ISC on the basis of mass (cover) values at increasing block sizes. KERSHAW [9] suggests that in such instances the correlation of two species requiring the same habitats is the same, viz. positive throughout since they respond to the environment in the same way at every measure of the scale. Two species with wider tolerance showing slight optimum differences may exhibit negative correlation at smaller scale measures (where the partial species pattern may differ according to a slighter variability of the environment) whereas positive correlation at larger scale measures [9]. Two species requiring entirely different habitats show negative correlation at every measure of the scale.

Most authors neglect interpreting the biological background of the different cases of correlation. The investigator is mostly on the horns of a dilemma

whether to attribute correlations to the effects of external, abiotic, physical reasons or to properties of the plant somehow associated with competition; in fact — apart from extreme cases — both factors most be reckoned with.

KOLBE [11] suggests that mass values of plant species reflect constitutional properties, and, on the other hand, mass relations (rather than presence–absence relations) are modified by influences of the habitat all, these resulting in what KOLBE calls "Verdrängungskraft", a means of competition which is materialized in the mass of the plant [16].

KERSHAW [10] e.g. attributes the negative correlation between *Festuca rubra* and *Carex bigelowii* to the performance of the latter species and to its direct effect on *Festuca rubra* rather than to an effect of the similarly conspicuous topography. On the other hand, interpreting the lack of correlation, ANDERSON [1] rather suspects a role of environment, suggesting together with GOODALL that on a scale determined by the environment, several factors can give rise to the species pattern. Under these circumstances, the lack of correlation between the species is not surprising.

It is obvious that the competition for light, water, dissolved nutrients, of populations forming a plant community cannot be left out of consideration when the correlations of species-pairs are interpreted [4, 16].

It may be supposed, however, that there is not in the least competition and struggle among all of the species-pairs. It may happen that the complementary compensatory properties (e.g. a root system penetrating deep or phaenophases different from those of population A) of a population B may "prevent" the nutrient or water draining effects of an aggressive competitor population A (e.g. spreading extensively in vegetative way). In such a case, a factor controlling both populations in space may successively bring them together so that a positive correlation develops.

At basic unit of our material, there are several cases of positive significant ISCs between species-pairs where one of the partners (e.g. *Oryzopsis* or *Vicia*) is extensively spreading vegetatively with individuals migrating horizontally in space and the others (e.g. *Dictamnus* or *Lathyrus*) having pole-like roots penetrating deep and appearing on the surface with one or two shoots only (Table 1). In these cases, no mutual exclusion occurs and positive correlation develops.

Positive significant ISC exists between *Polygonatum latifolium* and *Vicia* in various dimensions. Their ability to compensate each other's influences at smaller block sizes (those of two units) is preserved also at larger block sizes.

Within block sizes of one and two units, *Melittis* shows positive correlations with 8 species. Its strong affinity among others to species with aggressive compact spreading (e.g. *Lithospermum* and *Vicia*) gives an acompetitive character to this species (Table 1).

The case of positive correlation mentioned by KERSHAW (similar requirement for habitat) possibly holds for the *Melittis–Geum* species-pair at block sizes of 1, 4, and 16 basic units.

The negative correlations in species-pairs *Oryzopsis–Vicia*, *Brachypodium–Oryzopsis* and *Brachypodium–Lithospermum* consisting of individuals of large stretch, may reflect competition between strong competitors in blocks of one basic unit (Tables 2 and 4). The negative correlation between *Lithospermum* and two *Polygonatum* species in basic units may also represent competition. *Lithospermum* is in bloom and in the phase of seed formation in May and June while in the same period, *Polygonatum latifolium* and *P. odoratum* develop rhizome segments for the next year and roots on these segments. All of these three species, though being in different phaenophases, are in phases of intensive nutrient uptake during the same period of time.

In cases of correlations of alternating tendency where positive correlations exist at small block sizes, the similar habitat requirement of the species-pair may still be expressed, at larger block sizes, however, the heterogeneity of the sample plot and the variability of the habitat factors may result in negative correlations. This is the case in species-pairs of e.g. *Lithospermum–Melittis*, *Lithospermum–Dactylis*, and *Lithospermum–Lapsana* (Table 3).

Species-pairs showed significant correlation at two adjoining block sizes on a total of 20 cases. In 10 of these cases a reverse of the sign occurred. A change over from negative to positive correlation took place frequently: in *Lathyrus–Dactylis* and *Oryzopsis–Dactylis* shifting from single block-size to double block-size; in *Oryzopsis–Melittis*, *Lithospermum–Brachypodium* and *Geum–Lapsana* from double to quadruple block-size and in *Oryzopsis–Brachypodium* from block of 16 units to that of 32 units. (The latter species-pair showed negative correlation in each of the smaller blocks while positive one in blocks of 32 units, giving a counter-example for the *Lithospermum–Melittis* species-pair (Table 4).

It seems possible in such cases that the competition and struggle expressed in the long run in the cover values of species-pairs with similar requirements, are more emphasized in the small blocks; in the larger blocks, parallel to the fading of competition, the similarities in habitat requirements are rather manifested.

It may be mentioned here that KERSHAW [7, 8] himself has demonstrated that in the *Dactylis–Lolium* species-pair (at basic units of 80 cm), low cover values of the species result in positive correlation while at high shoot densities, negative correlation develops.

The results can be evaluated also according to increasing block sizes. The number of significant ISCs decreases from basic unit to quadruple block-size, increases from block size of 8 units to that of 16 units while stays at an unchanged level at the two highest block sizes. With regard to the shape and

orientation of the block and the number of correlation, a connection though not unidirectional seems to exist.

As to the sign distribution, either negative or positive significant ISCs may be dominant from block size of basic unit to that of 8 units. From block size of 16 units (64 m^2) upwards, the signs are distributed fairly evenly (Table 5).

It is striking that of the 12 significant ISCs in the block size of 4 units, 10 are positive and only those of *Lithospermum–Geum* and *Lithospermum–Polygonatum odoratum* are negative. Consequently, 16 m^2 areas of the sample plot are favoured with regard to the positive correlations of the stressed species.

Considering the number of a significant ISCs, the 12 species can be divided into 3 groups (Table 6). In the affinity order of the species, *Lithospermum* shows the highest while *Dictamnus* exhibits the lowest affinity.

In an attempt to deduce coalition correlatedness of the 12 species from elementary correlations, the network of correlatedness was drawn on the basis of the significant ISCs. The 3 species viz., *Vicia*, *Lathyrus* and *Oryzopsis* showing correlations in basic units most frequently, were in correlation with one another, too, and thus served as starting points or centres (polycentre) for other correlations. A strong spatial reticulum was characteristic within which every species was in correlation with at least two species (Fig. 1a).

In double blocks (Fig. 1b), species already showed a slight polarization in the spatial reticulum. Compared to *Melittis* favoured in the spatial reticulum with its 8 significant ISCs, *Oryzopsis* and *Lithospermum* lost significance as subcentres. *Vicia* and *Lathyrus* disappeared as centres of correlations and *Lithospermum* formed correlations mainly with species other than before.

Significant ISCs in quadruple blocks (16 m^2) formed the most ordered reticulum also from the geometrical point of view (Fig. 1c). Within the reticulum, the direct connection between two centres, *Lithospermum* and *Melittis*, had become discontinued. The correlations of *Melittis–Lathyrus*, *Melittis–Oryzopsis*, *Lithospermum–Brachypodium* and *Lithospermum–Geum* were the remnants of those developed at the preceding block-size. However, a breaking off of the reticulum and the formation of side chains indicating only one correlatedness, were characteristic when compared to those found in double blocks. Similarly, it was also for the first time obvious that the species of the *Vicia–Dictamnus* pair formed correlation with one another only detaching from the thus far contiguous reticulum. The total number of significant ISCs showed an abrupt decrease (to 12).

In blocks of 8 units, the reticulum of correlations was still fairly simple and a strengthening of a tendency to form chain-like correlations could be observed (Fig. 2a). The species clustered into two groups connected axis-like by species *Dactylis–Polygonatum latifolium–Lathyrus–Vicia*. The two groups consisted of species-pairs reticulated in the form of a triangle and a

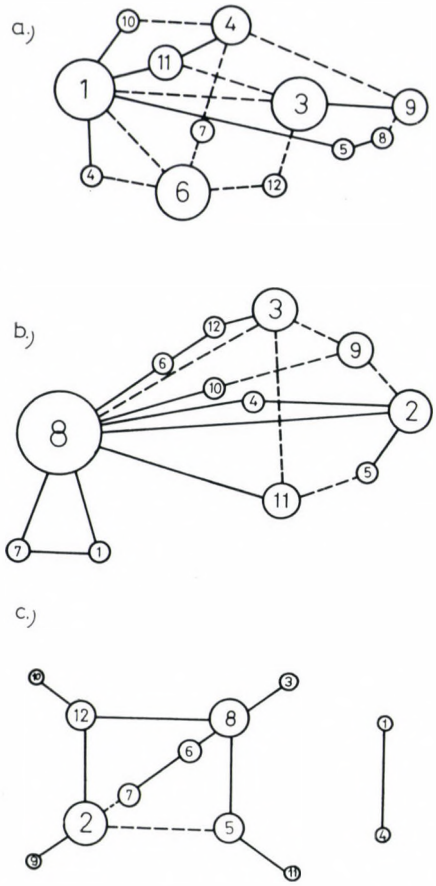

Fig. 1. Spatial reticulum of the correlations for the 12 species studied at block sizes of a = one unit, b = two units, c = four units. 1. *Vicia sparsiflora*, 2. *Lithospermum purpureo-coeruleum*, 3. *Oryzopsis virescens*, 4. *Dictamnus albus*, 5. *Geum urbanum*, 6. *Lathyrus niger*, 7. *Polygonatum latifolium*, 8. *Melittis grandiflora*, 9. *Brachypodium pinnatum*, 10. *Polygonatum odoratum*, 11. *Lapsana communis*, 12. *Dactylis glomerata*. Continuous and broken lines show positive and negative ISCs, respectively. The diameters of the circles are in direct proportion with the number of the correlations of the species

quadrangle at the ends of the axis. It was still *Lithospermum* and again *Lathyrus* that formed the centre of the latter group. *Lithospermum* was in correlation mostly with new species when compared to the previous block. *Vicia* which lost contact with the correlation reticulum of the previous block was again incorporated into the chain of correlations showing at its other end species with negative correlations only. *Polygonatum odoratum* formed no significant ISCs.

Block of 16 units represents a new organization. Relations of a new combination in most species and a pyramid-shaped spatial reticulum of

correlations developed (Fig. 2b). This form could be considered polycentric. The *Vicia–Lathyrus–Polygonatum latifolium–Dactylis* axis fastening the correlation reticulum together in blocks of 8 units lost contact with the reticulum and formed an independent chain of correlations. *Lithospermum* — besides its correlations already found in the previous block (*–Lapsana*, *–Melittis*) — again developed correlations with other species and thus through its

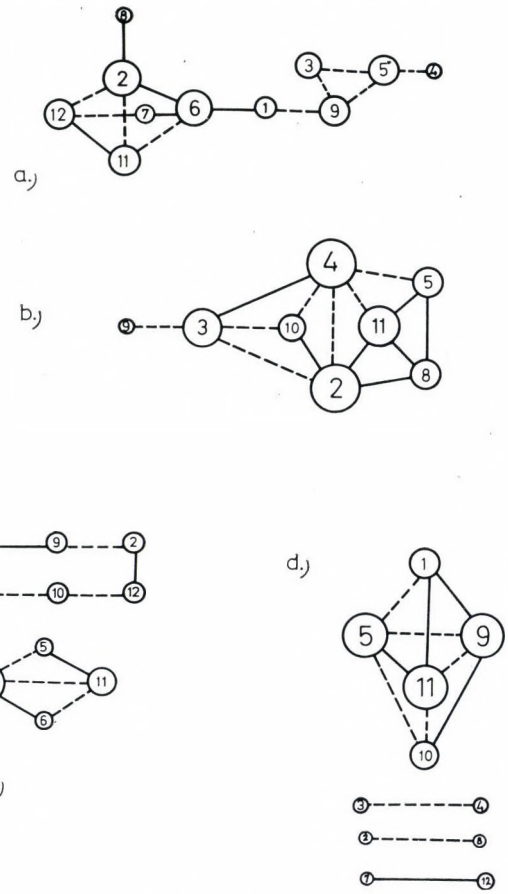

Fig. 2. Spatial reticulum of the correlations of species studied at block sizes of a = 8 units b = 16 units, c = 32 units, d = 64 units (continuation). For further explanation see Fig. 1

significant ISCs with *Oryzopsis* and *Dictamnus*, closed the open correlation chain of the block of 8 units.

In blocks of 32 units, 10 species were again — mostly indirectly — correlated. Of these, 4 showed reticulum-like and 6 exhibited chain-like correlations (Fig. 2c). It was *Polygonatum latifolium* and *Lapsana* that formed the centres of the geometrical configuration. Taken as a whole, correlations had

become simpler and decreased in number (significant ISC: 11). *Lithospermum* lost its central role in correlations here. *Brachypodium* and *Oryzopsis* were again situated at the end of the chain. *Melittis* and *Dictamnus* showed no correlation at all.

In blocks of 64 units, organization assumed the shape of a pyramid again. However, 3 species-pairs showing significant ISC within the pairs only had completely lost contact with the pyramid (Fig. 2d). *Lathyrus niger* showed no correlation.

It may be concluded that the strongest reticulum-like correlations reflecting multidirectional associations of the species are present in blocks of one and two units, by all means indicating a sort of organization of correlations [3] (see ANDERSON [1] who uses the term "complex social organization" for developed ISCs in the microstructure of the community). A simplification of the multilateral correlations among species as well as species-pairs and even species-quartets becoming independent of the other, represents an unambiguous tendency at increasing block sizes. At smaller block sizes, an actual contiguity, equal habitat requirements, competition etc. viz. more or less "real" ISCs exist between species-pairs and the multidirectional mutual correlations may possibly be attributed to interactions. On the other hand, at larger block sizes, the chain-like organization of the ISCs or the breaking off of the reticulum may be attributed to the heterogeneity of the habitat factors present on the sample plot and of the repetition of the spatial parts of larger sizes of the habitat which offer a common chance to live for some populations otherwise living in no tight connection.

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Acta biol. Acad. Sci. hung., **24**(1—2), 43—50 (1973)

STUDIES ON FAST AND SLOW GROWTH IN FUNGI

I. COMPARATIVE GROWTH OF CUNNINGHAMELLA ECHINULATA AND CLADOSPORIUM HERBARUM AS AFFECTED BY CULTURAL CONDITIONS

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(Received 1972—06—27)

Abstract

Cladosporium spores failed to germinate within 2 hours and never reached 100% germination at the end of a 18-hour period of cultivation on natural or semisynthetic medium. *Cunninghamella* spores, on the other hand, had germinated in nearly 100% by the end of the 2nd hour. Small doses of indoleacetic acid (5 parts/10^6) stimulated the growth and spore germination of both fungi. Gibberellin (at 5 parts/10^6) stimulated the growth of *Cunninghamella* only, and promoted the spore germination of both fungi. On agar medium, $NaNO_3$ was the best nitrogen source for growth, while on liquid medium, asparagin was superior to nitrate or ammonium carbonate. Although *Cunninghamella* mats reached maximum dry weight much earlier than *Cladosporium* mats, yet the maximum mycelial dry weight of the latter fungus exceeded that of the former. On natural media both fungi grew more intensively, but the above differences in their growth were the same in natural and semisynthetic media.

Introduction

According to COCHRANE [5] growth cannot be defined precisely; a provisional operational definition is increase in either mass or number of cells.

The factors affecting fungal growth are various and numerous, being usually classified into internal and external factors. The internal factors include the genetic constitution and the internal modification due to age. The external factors comprise temperature, pH, oxygen, CO_2, humidity and the composition and concentration of food. While more is known about the external factors, it remains beyond any doubt that the external environment acts by modifying the internal environment. The genetic constitution or inherent factors are the skeleton which find expression only when the environment is suitable.

The first phase of growth in fungi may be long or short depending upon the age and vigour of the inoculum, the medium and other factors [15]. The spore germination during this phase represents the first stage of growth. Not all nutrients are necessarily present in the spore, and different types of relation to external nutrients may therefore be expected, ranging from complete independence to complete dependence. The more abundant growth on natural media than on synthetic ones [2] is due to the complexity of the former and their richness is essential metabolites.

Since the discovery of auxin and its identification as a growth hormone, its effect on the growth of higher plants in particular, has been investigated by

many authors. Such effect involves cell elongation [3]; increase in osmotic solutes [4]; increased permeability to water [16]; reduction in wall pressure due to increased plasticity [20]; increase in wall synthesis [6]; and induction of specific RNA and protein synthesis.

The aim of this study was to explain the differences in growth of fungi in the light of their metabolic activities and hormonal relations. Comparison of the growth of a fast growing and a slow-growing fungus under variable external cultural conditions, such as type of medium, source of nitrogen and inclusion of some growth substances, was the first necessary step in this study.

Material and method

Two fungal strains, both isolated from Egyptian soil samples on Dox agar plates were used throughout. They were identified according to GILMAN [9] as *Cunninghamella echinulata* and *Cladosporium herbarum* respectively.* The *Cunninghamella* strain grows clearly faster than *Cladosporium* regarding either the time of its appearance on the plate or its spread on the surface of the nutritive medium.

Spore suspensions from 7-day-old slant cultures were used as inocula. The plates were incubated at 25 °C and duplicate samples were used throughout.

Results

Growth in different media

Three types of media were used namely, Dox agar (semisynthetic), potato extract agar and malt extract agar (natural). The radial growth of *Cunninghamella* was evident from the first day on, and this fungus covered almost the whole plate surface in 6 days. Surface spread of *Cladosporium* became measurable, irrespective of the medium, by the end of the 2nd day and it reached almost 2/3 of the dish by the end of the 14th day.

Daily radial growth (in cm) of both fungi on the three culture media during the first 6 days of the experiment are shown in Table 1. The daily growth revealed by the slow-growing *Cladosporium*, on any medium during each time interval, hardly exeeded 50% that reached by the fast-growing *Cunninghamella*. For one and the same fungus, and mainly during the first 2 days, the rate of growth was higher on the natural media than on the semi-synthetic one.

Growth substances

Three concentrations of each of indoleacetic acid (IAA) and gibberellin (GA) namely, 1, 5 and 10 parts/10^6 in Dox agar medium were used. Tryptophan was added in amounts equivalent to 4, 8 and 12 mg N/100 ml medium. These

* Their identity was confirmed by Prof. A. H. MOUBASHER (Assiut University, Egypt), to whom the authors are greatly indebted.

Table 1

Daily radial growth of Cunninghamella and Cladosporium colonies on the various culture media

Fungus	Medium	Growth in cm on the					
		1st day	2nd day	3rd day	4th day	5th day	6th day
Cunninghamella	Dox(control)	1.5	1.3	1.6	1.8	1.3	1.5
	Potato	1.9	1.9	1.5	1.3	1.1	1.3
	Malt	1.8	1.7	1.5	1.2	1.5	0.3
Cladosporium	Dox(control)	0.0	0.7	0.5	0.4	0.4	0.5
	Potato	0.0	1.1	0.6	0.6	0.4	0.6
	Malt	0.0	1.1	0.4	0.7	0.5	0.7

chemicals were added to the previously autoclaved medium under aseptic conditions.

Table 2 shows the daily growth of the colonies of both fungi, during the first 6 days, in absence or presence of IAA, GA or tryptophan. *Cladosporium* did not show measurable growth by the end of the 1st day, and in the following days the daily increase in its diameter was less than half the increase in the *Cunnunghamella* colony diameters. The daily linear growth was higher when *Cunninghamella* was cultivated in the presence of 5 parts/10^6 IAA, reaching its maximum on the 5th day. Some stimulation of growth was also evident in case of *Cladosporium*, though appearing somewhat later (6th day). In the presence of 5 parts/10^6 GA the daily linear growth of *Cunnighamella* colonies slightly

Table 2

Daily radial growth of Cunninghamella and Cladosporium under various treatments

Fungus	Medium	Rate of growth during					
		1st day	2nd day	3nd day	4th day	5th day	6th day
Cunninghamella	Dox (control)	1.5	1.4	1.6	1.7	1.3	1.5
	Dox + 5 parts/10^6 IAA	1.4	1.8	1.7	1.9	2.0	—
	Dox + 5 parts/10^6 GA	1.7	1.4	1.6	1.9	1.6	—
	Dox + 12 mg N tryptophan	1.3	1.4	1.8	1.3	0.7	1.5
Cladosporium	Dox (control)	0.0	0.7	0.5	0.4	0.4	0.5
	Dox + 5 parts/10^6 IAA	0.0	0.6	0.6	0.4	0.3	0.8
	Dox + 5 parts/10^6 GA	0.0	0.7	0.5	0.4	0.4	0.5
	Dox + 12 mg N tryptophan	0.0	0.6	0.6	0.4	0.2	0.5

exceeded the control values on the 5th day. The growth of *Cladosporium*, on the other hand, remained approximately constant. Addition of tryptophan to Dox agar medium reduced the growth of *Cunninghamella* on days 4 and 5, but did not affect the growth of the *Cladosporium*.

Source of nitrogen

Three N sources, viz., sodium nitrate, ammonium carbonate and asparagin were used; amounts equivalent to 33 mg N were added to 100 ml medium. Growth was measured by the linear method as well as by measuring the mycelial dry weight. Table 3 reveals a rapid appearance of measurable growth on the 1st day in case of *Cunninghamella* and that this fungus spread on the surface of the media faster than *Cladosporium*. The colony diameters can be arranged in the order of $NaNO_3 > (NH_4)_2CO_3 >$ asparagin for *Cunninghamella* and $NaNO_3 >$ asparagin $> (NH_4)_2CO_3$ for *Cladosporium*.

The superiority of $NaNO_3$ for growth of both fungi on Dox agar was not valid for the Dox liquid medium. The mycelial dry weights obtained on the latter medium were the highest when asparagin was used as nitrogen source. It should be mentioned in this respect that the initial pH value of the media sustaining growth of both fungi (pH 5.5) varied within normal limits (pH 4.8—6.2).

The mean values for the dry weight of mats (in mg) of both fungi on asparagin as N source are presented in Fig. 1. *Cunninghamella* reached its maximum growth much earlier (on the 4th day) than *Cladosporium* (on the

Table 3

Radial growth of Cunninghamella and Cladosporium when cultured on Dox agar medium containing different nitrogen sources
(in cm)

Fungus	Nitrogen source	Colony diameter at the end of					
		1st day	2nd day	3rd day	4th day	5th day	6th day
Cunninghamella	$NaNO_3$	1.5	2.8	4.4	6.2	7.5	9.0
	Asparagin	0.9	1.8	3.1	4.5	5.5	7.1
	$(NH_4)_2CO_3$	1.0	2.0	3.7	5.4	6.6	7.6
Cladosporium	$NaNO_3$	0.0	0.7	1.2	1.6	2.0	2.5
	Asparagin	0.0	0.7	1.0	1.5	2.1	2.4
	$(NH_4)_2CO_3$	0.0	0.6	1.2	1.3	1.7	2.2

L. S. D. for *Cunninghamella* at 0.05 level = 0.18
at 0.01 level = 0.27
L. S. D. for *Cladosporium* at 0.05 level = 0.04
at 0.01 level = 0.06

Table 4

Percentage germination of Cunninghamella and Cladosporium spores by the end of different periods of time following inoculation

Dox medium enriched with	Fungus	% spore germination at end of (hour)							
		1	2	3	6	9	12	15	18
none	*Cunn.*	71	100	100	100	100	100	100	100
(control)	*Clad.*	—	—	3	8	26	44	56	72
5 p.p.m.	*Cunn.*	100	100	100	100	100	100	100	100
IAA	*Clad.*	—	—	6	10	17	51	57	80
5 p.p.m.	*Cunn.*	79	100	100	100	100	100	100	100
GA	*Clad.*	—	—	7	16	19	47	68	92
12 mg N	*Cunn.*	85	97	100	100	100	100	100	100
in tryptophan	*Clad.*	—	—	12	27	28	52	56	81

L. S. D. at 0.05 for *Cunninghamella* = 4%
at 0.05 for *Cladosporium* = 3%
at 0.01 for *Cunninghamella* = 6%
at 0.01 for *Cladosporium* = 5%

12th day). It should be noted, however, that the mycelial dry weight reached by *Cladosporium* was higher than that reached by *Cunninghamella* during its shorter growth period.

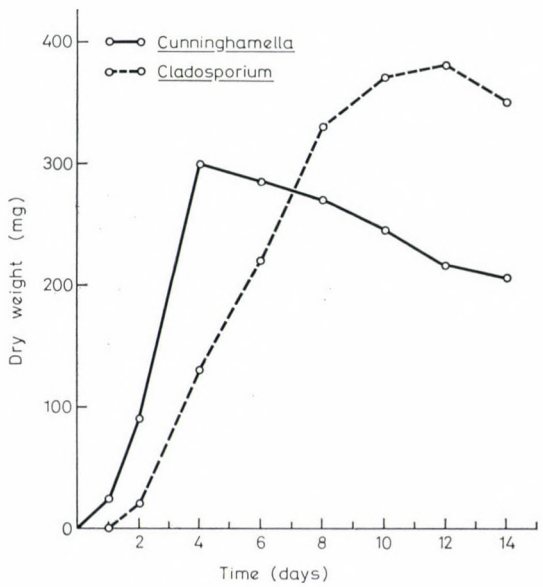

Fig. 1. Dry weight of mats (in mg) of *Cunninghamella* and *Cladosporium* raised on Dox-liquid medium containing asparagin for 14 days

The appearance of measurable growth in case of *Cunninghamella* one day prior to *Cladosporium* was a repeated observation during this work. Since spore germination is the first step in growth, the percentage spore germination was estimated for both fungi on asparagin containing Dox liquid medium enriched with 5 parts/10^6 IAA, 5 parts/10^6 GA or 12 mg N as tryptophan. As inoculum, equal numbers of spores were used from each fungus. Table 4 shows that *Cladosporium* spores failed to germinate during the first two hours while those of *Cunninghamella* germinated in almost 100%. By the end of the experiment (18 h) the percentage spore germination of *Cladosporium* was still less than 100%. Variation in the composition of the medium seemed to influence spore germination significantly.

Discussion

Many fungi have latent abilities to synthesize various essential metabolites and it is a common experience to find that a trace of some crude natural product stimulates the rate of growth, sporulation and spore germination of a fungus [7, 18, 21]. The superiority of natural to synthetic media for growth appears to be due to the complexity of the natural media [2].

The present work revealed significantly higher growth of *Cunninghamella* and *Cladosporium* on the two natural media used, especially on potato extract-agar. Growth stimulation on potato agar was more evident in the case of the slow-growing *Cladosporium*. In spite of this, the great difference in growth rate and germination time between the two fungi was striking on natural media as well. As regards germination, HAWKER and co-workers [11] reported that *Cunninghamella elegans* showed conidial germination from 1 to 3 h after being placed in water or nutrient solution. The spores of our *Cunninghamella* and *Cladosporium* strains began to germinate in the first and third hour, respectively.

The data presented here lead to the suggestion that the differential growth rates of both fungi might be due to some other factors than nutritional, most probably hormonal, or, in other words, genetical.

It is now recognized that most, if not all, of the physiological activity of the plant is regulated by a variety of chemical substances, i.e., hormones. Accumulative data describing the effect of IAA and its allies are available, most of which are on higher plants. In some cases the effect is stimulatory, in others inhibitory and in still other cases IAA is a necessary participant in the growth activity of other plant hormones, viz., cytokinins and gibberellins [10, 12—14].

The present investigation revealed an increased daily growth of both *Cunninghamella* and *Cladosporium* in the presence of 5 parts/10^6 IAA, reaching its maximum earlier in the former than in the latter fungus. Significant

growth stimulation on Dox agar medium was only shown by *Cunninghamella* in the presence of 5 parts/10^6 GA. Moreover, inclusion of tryptophan in the Dox agar medium resulted in a late slight suppressive effect on the growth of *Cunninghamella* whereas the growth of *Cladosporium* was unaffected by tryptophan.

Superiority of one source of nitrogen over others for fungal growth, varies with different fungi. Thus, KNO_3 and asparagin were reported to be better than $(NH_4)_2SO_4$ for growth and sporulation of *Fusarium udum* [17]. Excellency of asparagin as compared to other nitrogen sources for fungal growth was found by WILLIS [22], AUBE and AGNON [1] and STEPHEN and CHRISTINA [19].

The present work revealed that the growth of the two fungi under study varied not only with the nitrogen source but also with the nature of the nutritive medium. On solid medium, both fungi preferred $NaNO_3$ to $(NH_4)_2CO_3$ or asparagin. On the other hand, higher mycelial dry weights of both fungi were produced on liquid medium enriched with asparagin than with $NaNO_3$ or $(NH_4)_2CO_3$. That one method of measuring growth may disagree with another was claimed by FRIES [8] and LILLY and BARNETT [15].

The present work also showed that maximum growth (in dry weight terms) of *Cunninghamella* was reached much earlier than that of *Cladosporium*; after the peak of growth signs of autolysis began to appear. This behaviour indicates that *Cunninghamella* draws its growth curve in a faster rate than the slower *Cladosporium*. Nevertheless, *Cladosporium* reached, during its optimum period of growth, a higher mycelial dry weight than that produced by *Cunninghamella* during its own respective period. COCHRANE [5] stated that dry weight in the filamentous fungi may be deceptive inasmuch as it can represent in part the accumulation of polysaccharides, lipids or wall materials without increase in living protoplasm. Whether this is true or not, will be the subject of a forthcoming investigation.

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Acta biol. Acad. Sci. hung., **24**(1—2), 51—57 (1973)

STUDIES ON FAST AND SLOW GROWTH IN FUNGI

II. COMPARATIVE GROWTH OF CUNNINGHAMELLA ECHINULATA AND CLADOSPORIUM HERBARUM IN THE LIGHT OF THEIR CARBOHYDRATE AND NITROGEN METABOLISM

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(Received 1972—06—27)

Abstract

The differences in the maximum dry weight between the two fungi under study seems to be a false criterion of difference in growth, because it represents differences in accumulated amounts of polysaccharides rather than proteins. The slow growth of *Cladosporium* may be partly due to its higher ammonium content as compared to *Cunninghamella* hyphae. *Cunninghamella* absorbed greater amounts of tryptophan and revealed higher efficiency in transforming most of the absorbed amounts into other compounds.

Introduction

Methods of measuring fungal growth are numerous and well-known, but there is no general method to be recommended for all. The most widely used method is estimation of growth by determination of the dry weight of the mycelium. However, dry weight may reflect accumulation of polysaccharides, lipids, or wall material rather than synthesis of new protoplasm [2]. Oxidative assimilation is often very active in fungi under conditions which restrict protein synthesis [1, 4, 14, 16].

The potentialities of a fungus to utilize different sugars or nitrogen compounds and to synthesize various essential metabolites before it can grow are limited by its genetic constitution.

In a previous work the authors [13] studied the effect of some cultural conditions on the growth rates of *Cunninghamella echinulata* (fast-grower) and *Cladosporium herbarum* (slow-grower). They found that the former fungus reaches its optimum period of growth much earlier that the latter. The maximum of the mycelial dry weight was higher in case of *Cladosporium* as compared to *Cunninghamella*.

The objective of the present work was a trial to interpret growth differences between *Cunninghamella* and *Cladosporium* in relation to their carbohydrate and nitrogen metabolism.

Material and method

As growth medium for control cultures Dox liquid medium with asparagin as N source was used [13]. Additional ingredients 5 parts/10^6 indol acetic acid (IAA), 5 parts/10^6 gibberellin (GA) or tryptophan corresponding to 12 mg N(100 ml) were added to the sterilized medium.

Each flask contained 50 ml medium. Spore suspensions from 7-day-old slant cultures were used as inoculum. The inocula contained equal number of spores. Each combination was prepared in duplicate. The cultures were incubated at 25 °C for 10 days during which samples were taken for carbohydrate and nitrogen analysis of media and mats at 48 and 144 h and at the end of the incubation. The methods used for extraction of media and mats were those recommended by NAGUIB [9]. All the carbohydrate and nitrogen fractions were estimated colorimetrically. NELSON's method [11] was that adopted for determination of sugar in terms of glucose (DRV, TRV, and polysaccharides). Ammonia-N was determined according to DELORY [5], amino- and protein-N according to RUSSELL [12], amide-N according to NAGUIB [10], nitrate-N according to TAHA [18], peptide-N according to SUTHERLAND and co-workers [17], and tryptophan according to SNELL and SNELL [15].

Results

Dry weight of mats

Table 1 shows that the two fungi responded in a different manner to the additional chemicals. Thus, the dry weight of the *Cunninghamella* mats that had been cultivated to 2 or 6 days in presence of IAA, GA or tryptophan were below the control values. In the 10-day samples the differences were less expressed. On the other hand, IAA and GA failed to influence the growth of *Cladosporium*. The 6-day values for the tryptophan-containing cultures were significantly lower than the control values of *Cladosporium*.

The growth behaviour of *Cunninghamella* and *Cladosporium* on control Dox medium revealed significantly higher dry weights of the former fungus after 2-day and 6-day incubation. However, by the end of the incubation the *Cladosporium* strain exceeded the *Cunninghamella* in mycelial dry weight.

Analysis of media

Carbohydrate analysis: The total sugar uptake/mat of *Cladosporium*, in each comparison, was slightly higher than the respective values reached by *Cunninghamella*, except in presence of GA (Table 2). Since such differences may be attributed to the corresponding differences in dry weight of mats, the total sugar uptake/1 g of the final dry weight of each fungus was calculated. This value was, in each comparison, higher in *Cunninghamella* than in *Cladosporium*, especially in the presence of IAA or tryptophan.

Nitrogen analysis: The results of nitrogen analysis are summarized in Table 3. The total amounts of nitrogen absorbed by the two fungi were almost equal at the end of the experiment (10 days). Both fungi, irrespective of treatment, preferred the amide-N to the amino-N of asparagin.

Analysis of mats

Nitrogen analysis: In the mats nitrogen was determined in different forms viz., nitrate, ammonia, amino, amide, peptide, protein, free tryptophan and bound tryptophan. The values of the soluble N (mg N/g final dry weight) were of limited variations in both fungi, except for the ammonia-N. The latter

Table 1

Mean values of dry weight of mats of Cunninghamella and Cladosporium at the end of three growth periods under various treatments
(in mg)

Dox liquid medium enriched with	*Cunninghamella*			*Cladosporium*		
	2 days	6 days	10 days	2 days	6 days	10 days
none	119	296	278	29	241	357
5 parts/10^6 IAA	46	188	237	31	241	373
5 parts/10^6 GA	59	248	295	30	256	350
12 mg N as tryptophan	22	187	240	15	158	370

L. S. D. at 0.05 level = 39
at 0.01 level = 52

Table 2

Mean values of total sugar uptake by Cunninghamella and Cladosporium mats grown for 10 days in variously treated Dox liquid medium (as mg sugar)
(in mg)

Medium enriched with	*Cunninghamella*		*Cladosporium*	
	Total/mat	Total/1 g final dry weight	Total/mat	Total/1 g final dry weight
none	1 570	5 652	1 630	4 565
5 parts/10^6 IAA	1 590	6 709	1 640	4 397
5 parts/10^6 GA	1 500	5 085	1 640	4 686
12 mg N as tryptophan	1 600	6 666	1 600	4 324

Table 3

Mean values of total nitrogen absorption (in mg N/mat/100 ml medium) from asparagin by Cunninghamella and Cladosporium mats grown for 10 days on differently treated Dox liquid medium

Medium enriched with	*Cunninghamella*			*Cladosporium*		
	Uptake from			Uptake from		
	Amino-N	Amide-N	Total	Amino-N	Amide-N	Total
none	15.9	17.0	32.9	15.8	17.0	32.8
5 parts/10^6 IAA	15.8	17.0	32.8	15.9	17.0	32.9
5 parts/10^6 GA	16.0	17.0	33.0	16.0	17.0	33.0
12 mgN as tryptophan	15.0	17.0	32.0	15.0	17.0	32.0

fraction was found in higher amounts in *Cladosporium* mats especially during the first two days, than in *Cunninghamella* mats.

Table 4 includes the average values of total soluble N (T.S.N.), protein-N, and total N (T.N.) (as mg N/1 g final dry weight). It shows that the additional

Table 4

Average values of total soluble nitrogen, protein and total nitrogen (as mg N / 1 g final dry weight of each period) in mats of Cunninghamella and Cladosporium raised for 3 growth periods under different treatments

Medium enriched with	Growth period (day)	*Cunninghamella*			*Cladosporium*		
		T.S.N.	Protein-N	T. N.	T.S.N.	Protein-N	T. N.
none	2	22.0	5.5	27.5	28.6	5.0	33.6
	6	19.4	6.5	25.9	16.8	18.0	34.8
	10	16.7	14.4	31.1	13.6	21.3	34.9
5 parts/10^6 IAA	2	23.5	17.1	40.6	28.8	6.1	34.9
	6	16.2	23.2	39.4	16.0	19.0	35.0
	10	14.9	23.1	38.8	13.9	20.6	34.5
5 parts/10^6 GA	2	20.2	18.6	38.8	30.0	4.5	34.5
	6	18.7	21.3	40.0	15.5	23.2	38.7
	10	16.8	18.8	35.6	14.8	24.0	38.8
12 mg N as tryptophan	2	23.4	13.0	36.4	25.6	7.6	33.2
	6	21.0	15.8	36.8	20.3	12.8	33.1
	10	17.8	16.4	34.2	13.8	20.4	34.2

ingredients in the medium resulted in no measurable variation in the T.S.N. content of the mats. In *Cunninghamella* the increase in protein-N was substantially accelerated by either of IAA, GA, and tryptophan in the medium. In the *Cladosporium* strain, on the other hand, the rate of protein synthesis was not influenced by these substances. On the whole, the differences between the respective nitrogen fractions in the two fungi are too small to account for the recorded differences in dry weights at the end of the experiment.

Table 5 includes the total tryptophan-N uptake from the medium and its amount as well as its percentage transformation in mats of both fungi by the end of the experiment. It shows that *Cunninghamella* absorbed more than double the amount of tryptophan-N taken up by *Cladosporium*. The latter fungus retained almost half of the absorbed tryptophan in a free unchanged form. *Cunninghamella* was more capable of transforming most of the absorbed tryptophan into other compounds. Tryptophan incorporated in protein could not be detected by the method applied by us.

Carbohydrate analysis

Table 6 includes the average values of hexoses and polysaccharides in mats of both fungi; sucrose was not included due to its negligible value. It shows that the polysaccharides in control mats of both fungi increased gradu-

Table 5

Average values of total tryptophan uptake from the medium, its amount as well as its percentage transformation in mats of Cunninghamella and Cladosporium after 10-day-growth on Dox medium containing tryptophan
(as mg tryptophan-N)

Medium enriched with	mg N per	*Cunninghamella*			*Cladosporium*		
		Uptake	in mat	% transformation	Uptake	in mat	% transformation
12 mg N as tryptophan	Mat	0.9	0.18	80	0.4	0.207	48.2
	1 g final dry weight	3.8	0.76	80	1.08	0.560	48.1

Table 6

Average values of hexoses and polysaccharides in Cunninghamella and Cladosporium mats raised on differently treated Dox medium for three growth periods
(as mg glucose/1 g final dry weight of each period)

Medium enriched with	Growth period (day)	*Cunninghamella*			*Cladosporium*		
		Hexoses	Polysaccharides	Total carbohydrate	Hexoses	Polysaccharides	Total carbohydrate
none	2	36.0	23.3	59.3	0.4	272.6	273.0
	6	0.6	31.9	32.5	14.0	291.2	305.2
	10	0.4	44.6	45.0	0.2	353.0	353.2
5 parts/10^6 IAA	2	57.6	48.9	106.5	1.2	137.9	139.1
	6	0.5	52.6	53.1	2.8	312.1	314.9
	10	1.8	35.8	37.6	0.5	384.5	385.0
5 parts/10^6 GA	2	5.2	31.8	37.0	0.9	171.4	172.3
	6	0.3	83.3	83.6	0.2	197.8	198.0
	10	1.7	54.4	56.1	0.2	322.7	322.9
12 mg N as tryptophan	2	10.4	23.0	33.4	0.3	12.4	12.7
	6	0.5	38.3	38.8	15.2	240.0	255.2
	10	2.5	16.8	19.3	0.4	360.3	360.7

ally till the end of the experiment. Various treatment of *Cunninghamella* mats enhanced the polysaccharide synthesis during the 1st and 2nd growth periods, after which a drop in this fraction occurred, denoting its probable breakdown during autolysis. The picture in *Cladosporium* was different, since the polysaccharide was always increasing indicating that signs of autolysis have not yet been reached till the end of the experiment. However, *Cladosporium* mats

revealed astonishing higher values of polysaccharides than *Cunninghamella*. Such differences are large enough to account for the higher mycelial dry weight of *Cladosporium* at the end of the experiment.

Discussion

The difference in dry weight appearing at the peaks representing the maximum growth of *Cunninghamella echinulata* and *Cladosporium herbarum* [13] seems to be a false criterion of growth. The present work revealed that variations in the levels of T.S.N., protein and T.N. in both fungi are too small to account for the mycelial dry weight differences. The polysaccharide content seems to be the major fraction responsible for such differences, which are consequently related to oxidative assimilation rather than to protein synthesis. Increase in protein, or nitrogen in general, is the real sign for increase in living protoplasm. COCHRANE [2] stated that mass-dry weight, usually — in the filamentous fungi may be deceptive inasmuch as it can represent in part the accumulation of polysaccharides, lipids or wall materials without any increase in living protoplasm.

From the present study, however, and in the light of the revealed metabolic activities of the two fungi under study, several points should be stressed.

Slow growth of *Cladosporium*, especially during the first growth period, may be partly attributed to its tendency to accumulate in its hyphae higher amounts of ammonia than those found in *Cunninghamella*. Ammonia in high concentrations is toxic [3, 6—8].

Although the total absorbed amounts of nitrogen from asparagin were almost equal in the two fungi at the end of the experiment, yet, higher protein values in the variously treated *Cunninghamella* as compared to *Cladosporium* mats were observed during the first growth period. This behaviour, if the real meaning of growth is not overlooked, indicates not only faster growth but also higher tendency of *Cunninghamella* to build up more protoplasm during its early growth.

The higher ability of *Cunninghamella* as compared to *Cladosporium* to absorb tryptophan, coupled with the higher efficiency of the former in transforming most of its absorbed amounts of this amino acid into another compound(s), focuses the light on the possible variability in both fungi to synthesize, naturally, indoleacetic acid.

The above-mentioned possibility will be the subject of our next work.

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Acta biol. Acad. Sci. hung., **24**(1—2), 59—64 (1973)

STUDIES ON FAST AND SLOW GROWTH IN FUNGI

III. HORMONAL PICTURE IN MEDIA AND MATS OF CUNNINGHAMELLA ECHINULATA AND CLADOSPORIUM HERBARUM

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(Received 1972—06—27)

Abstract

In the filtrates of *Cunninghamella echinulata* cultures growth promoters, in the *Cladosporium herbarum* mats growth inhibitors were found in well-demonstrable amounts. Both fungi produced the same types of gibberellins (GA_3 and GA_4 or A_9) in their growth media.

Introduction

Literature on the production of growth substances by fungi is limited. Thus, production of indoleacetic acid (IAA) by both pathogenic and non-pathogenic fungi has been reported by Allen [1], Mace [11], Gruen [9], Novat [13], Mahadevan and Chandramohan [12], Leal and co-workers [10], Beltra and co-workers [2] and Yu and co-workers [21].

Since the first isolation of gibberellin from *Fusarium moniliforme* Sheld (*Gibberella fujikuroi* (Saw.) Wr.), the search for it in other fungi has been overlocked; attention being generally focused on its effect on higher plants.

In a previous work the authors [16, 17] demonstrated that of the two fungi under study the *Cunninghamella* grew faster than the *Cladosporium*, under various cultural conditions. Moreover, the former transformed into other compounds a greater part of the tryptophan taken up from the medium as compared to the latter.

In the present work we attempted to interpret growth differences in both fungi in the light of their hormonal picture.

Material and method

Preparation of mats: Mycelial mats were obtained after 4-day growth at 25 °C on Dox liquid medium containing asparagin as N source. This age is most suitable, since it represents active growth in both fungi without signs of autolysis [16]. Filtration was followed by gentle blotting of mats between filter papers. On the basis of equal dry weights, different fresh weights from both fungi were extracted.

Preparation of filtrates: Filtrates of 7-day-old mats of *Cunninghamella* and *Cladosporium* grown at 25 °C on the medium characterized above were subjected to extraction.

Extraction, separation and bioassay: The methods used for extraction of growth substances, separation through paper (partition) chromotography and bioassaying of activity by means of *Hordeum coleoptile* cylinder test were those recommended by Foda and Radwan [5, 6, 7, 8]. Separation by thin-layer chromatography and detection of gibberellins (GA) were carried out according to Paleg [15].

Results

Mycelial mats

Definite amounts of the ethyl alcohol extract of *Cunninghamella* or *Cladosporium* mats were chromatographed; the chromatograms were developed in isopropanol : water (80 : 20 v/v). Some chromatograms were tested for biological activity, others were used for colour reactions.

Fig. 1 presents the biological activity of the different chromatogram strips. The extract of *Cunninghamella* mats showed no zones of biological activity. *Cladosporium* mats, on the other hand, showed one zone of growth inhibition but no zones of growth promotion. The zone of growth inhibition was at chromatogram strip No. 10 which correspond to R_f value from 0.9 to 1.0. The colour reaction appeared negative.

Filtrates

The ethyl alcohol extracts of media were chromatographed in isopropanol : water (80 : 20 v/v). The chromatograms were then tested for biological activity and colour reactions. It is clear from Fig. 2 that *Cunninghamella* filtrate showed two zones of growth promotion, but no zones of growth inhibition. The first zone was at chromatogram No. 4, corresponding to R_f value from 0.3 to 0.4. A green spot of R_f value (0.2 to 0.4) appeared after spraying the chromatogram with Salkowski reagent. At the same R_f value a pale pink colour appeared with Ehrlich reagent and a yellow colour with nitrous-nitric reagent.

The second zone of growth promotion was at chromatogram strips No. 6, 7 and 8, corresponding to R_f values from 0.5 to 0.8. Salkowski reagent revealed a yellowish green spot of R_f value (0.5 to 0.6). At the same R_f value an orange spot was obtained with Ehrlich reagent. Nitrous-nitric reagent produced a pale blue spot of R_f value (0.6—0.7).

It seems therefore possible to conclude that *Cunninghamella* filtrate contained two growth promoters, probably of indol nature.

On the other hand, *Cladosporium* filtrate showed no zones of biological activity. In the chromatogram Ehrlich reagent produced a pale red spot of R_f value (0.2—0.4) and a yellow spot of R_f value (0.4—0.5). Spraying the chromatogram with nitrous-nitric reagent resulted in a yellow spot at R_f (0.2—0.4) and a pink spot at R_f (0.4—0.5). It is interesting that some growth promoters, though non-significant at the 0.01 level, were detected at R_f values corresponding to the coloured spots.

On the whole, it is clear that the growth-promoting activity is greater in *Cunninghamella* than in *Cladosporium* media.

The thin-layer chromatograms used for the separation of gibberellins from extract of filtrates of the cultures of both fungi were dried in cold air

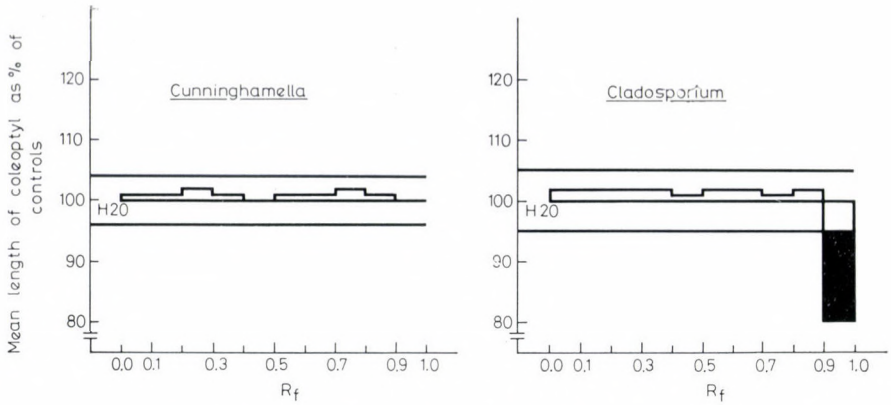

Fig. 1. Coleoptile test for fractionated alcohol extracts of *Cunninghamella* and *Cladosporium* mats

before being sprayed with ethanol/sulphuric acid (95 : 5). The plates were then heated at about 120 °C for 20 minutes, and the colour was carefully examined in ultraviolet light.

Table 1 includes the chromatographic properties of gibberellins found in the filtrate extracts. It shows that the medium of each of the two fungi revealed two spots on the chromatogram at 1.0 and 1.2 RGA (movement relative to gibberellic acid A_3). The produced colour, under ultraviolet light, was faint green-blue at RGA 1.0 and purple at RGA 1.2, which correspond to GA_3 and GA_4, or A_9, respectively.

It appears in the light of this qualitative test that both fungi are able to produce the same types of gibberellins.

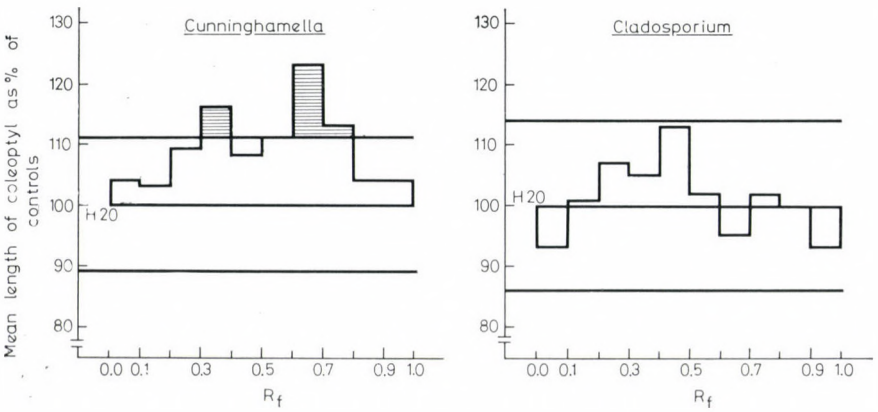

Fig. 2. Coleoptile test for fractionated alcohol extracts of media on which *Cunninghamella* or *Cladosporium* were grown for 7 days

Table 1

Thin-layer chromatographic properties of the gibberellins in the extracts of media on which Cunninghamella or Cladosporium were grown for 7 days

Item	*Cunninghamella*		*Cladosporium*	
	1st spot	2nd spot	1st spot	2nd spot
RGA*	1.0	1.2	1.0	1.2
Colour	Faint green to blue	Purple	Faint green to blue	Purple
Type of proper gibberellin	A_3	A_4 or A_9	A_3	A_4 or A_9

* Movement relative to gibberellic acid (A_3)

Discussion

It is now recognized that most, if not all, of the physiological activities of the plant are regulated by a variety of hormones. Growth is by far the most complex of all physiological processes.

The present work revealed, through the bioassay study of growth substances the existence of highly significant amounts of growth promoters, probably of indolic nature, in the medium sustaining *Cunninghamella* mats. These promoters could not be detected in considerable amounts in the growth medium of *Cladosporium*. This fact if added to the prevalence of growth inhibitory substances in *Cladosporium* hyphae and their absence in *Cunninghamella*, are most probably the clews which explain growth variability in the two fungi under study. Failure to detect growth promoters in mats of both fungi does not imply their absence there. This may be either to improper amounts of mats experimented with or to its production in the growing hyphal tips from where it becomes excreted into the external medium. The second possibility is more probable since SMITH [18, 19] and STADIER [20] stated that the fungal hypha grows in length by elongation of a zone just behind the tip. Further from the tip the wall thickens becoming rigid and is no longer capable of extension.

The natural production of IAA in higher amounts by *Cunninghamella* than by *Cladosporium* would explain why smaller doses of this growth regulator (5 parts/10^6) enhanced growth of both fungi when included in their Dox agar medium, while larger doses (10 parts/10^6) inhibited only *Cunninghamella* growth and failed to affect *Cladosporium* [16].

Limited literature on GA and auxin interaction suggests that they may act both independently and together, depending upon many factors. The fact that the condition of dwarfism can be corrected by application of GA is attrib-

uted by BRIAN and HEMMING [3] to GA deficiency. *Cladosporium*, as shown during the present work, could synthesize GA as *Cunninghamella* did, and still exogenous application of GA did not stimulate the limited surface spread of the former fungus [16]. Some authors believe that an excess of some natural inhibitors is present in dwarf plants, and that GA counteracts the effect of these [4]. This assumption does not match with the present findings, since counteracting the effect of inhibitors present in *Cladosporium* mats was not evident. OCKERSE and GALSTON [14] suggested that GA is dependent upon IAA for its action. Is the same valid for *Cladosporium*, or is slow growth a dominant character in this fungus? It would be premature to answer this question until more experimental results become available.

On the whole, it appears from the present study of SALAMA and co-workers [16, 17] that growth differences between *Cunninghamella* and *Cladosporium* are genetically controlled *via* production of growth promoters and inhibitors in various levels. Moreover, faster growth of *Cunninghamella* is, most probably, a matter of cell elongation rather than increase in dry matter.

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Acta biol. Acad. Sci. hung., **24**(1—2), 65—72 (1973)

EFFECT OF GAS MIXTURES OF VARIOUS O_2 CONCENTRATIONS ON FIBROBLAST CULTURES OF CHICK EMBRYOS*

IV. STUDIES ON HEXOSAMINE PRODUCTION

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(Received 1972—07—17)

Abstract

Fibroblast-like cells from 9-day-old chick embryos were cultured in the presence of gas mixtures containing 20, 7, 4 or 2% oxygen and the hexosamine content of the medium was determined after different periods of cultivation. The fibroblasts produced hexosamine in the presence of each gas mixture showing the highest production when 4% O_2 was present. This gas mixture represents a hypoxic milieu for the cells examined. Less hexosamine was produced with an oxygen concentration of 7% (normal O_2 content) or 2% (severe hypoxia). At a concentration of 20% the hexosamine production was of low degree and soon ceased. Selectively changed O_2 supply seems to affect the production of mucopolysaccharides as tested by hexosamine determination. The optimal concentration for biosynthesis is a medium moderately deficient in oxygen. In the initial stage the cells utilized hexosamine and no signs referring to their differentiation to cartilage could be observed.

Introduction

Investigation of the formation of acid mucopolysaccharides (MPS) has led to the elucidation of numerous details of their biosynthesis [10, 33, 36]. It has been generally agreed that the two basic components, viz. hexosamine (ha) and uronic acid, of the repeating unit of MPS originate from substances known as intermediaries of the Embden—Meyerhof pathway. In fact, ha has been described as originating from hexose-6-phosphates, while uronic acid from glucose-1-phosphate [4, 33, 34].

The presence of common substances participating in the Embden—Meyerhof pathway and biosynthesis of MPSs call the attention to the correlation between these processes. Nevertheless, their connection is little known. Some authors suggest that increased MPS production is concomitant to augmented glycolysis [9, 14], others claim that production of MPS results in utilization of the intermediaries and consequent narrowing down of the

* This work was supported by a grant from the Fonds National Suisse pour le Développement de la Recherche Scientifique

Embden—Meyerhof pathway [17]. The former suppositions are further complicated by observations difficult to interpret, e.g. it has been stated that of sarcomatous tissues displaying marked glycolytic activity, some (Rous sarcoma) do produce ha, others (Jensen sarcoma) do not [14].

By investigating the causal factors of MPS production, it is of essential importance that tissues poorly supplied with blood contain greater amounts of MPS than richly vascularized tissues [5]. Poor vascularization seems to provide favourable circumstances for MPS biosynthesis [17, 24]. One of the consequences of poor vascularization may be a condition of relative O_2 deficiency and in this sense it is reasonable to suppose that a correlation may exist between O_2 deficiency and MPS production. In decreased vascularization, however, in addition to "insufficiency" of oxygen supply, reduction in further essential functions (food supply, transport of metabolic by-products, constant internal environment, etc.) may occur. Therefore, under *in vivo* conditions, accumulation of MPS may be determined, in addition to O_2 deficiency, by further factors. *In vitro* conditions in a closed space are favourable for production of specific O_2-deficient environment. (In a closed space the essential conditions of cells and tissues can be ensured and by maintaining the other conditions unchanged it is possible to change the quantity of the components of the gas mixture including oxygen.)

The purpose of our investigations was to obtain data on the connection between O_2 supply and MPS formation, using relatively simple *in vitro* systems.

Material and method

Investigations were conducted on primary cultures of fibroblasts of connective tissue origin obtained from 9-day-old chick embryos. The method as described in detail elsewhere [18] is based on the procedure of Moscona [30]. Disperged cells, 250 000/ml were suspended in a medium composed of Hanks solution containing 0.5% lactalbumin hydrolysate (Difco), 90% and normal horse serum, 10%. To this medium 50 units of penicillin and 50 μg streptomycin per ml were added. The cell suspension was distributed in T-flasks (Bellco T 60), 17.5 ml/flask. The flasks were tightly closed and placed in a thermostat (37 °C) for 24 h. Then the medium was decanted and put aside for examination. The cells in the flask were washed with 5 ml CMF-PBS (pH 7.4) at room temperature and supplied with the following medium:

Earle saline containing 0.5% lactalbumin hydrolysate (Difco) and 0.1% yeast extract (Difco), 90% and normal horse serum, 10%; antibiotics added as above.

After exchange of medium the flasks were closed by rubber stoppers supplied with metal canules through which gas was introduced in them. The following gas mixtures were used;

20% O_2 + 75% N_2 + 5% CO_2	152.0 mmHg pO_2
7% O_2 + 88% N_2 + 5% CO_2	53.2 mmHg pO_2
4% O_2 + 91% N_2 + 5% CO_2	30.4 mmHg pO_2
2% O_2 + 93% N_2 + 5% CO_2	15.2 mmHg pO_2

Description of the procedure of gassing has been given in detail in a previous work [18].

The cultures were removed and processed on the days 1, 3, 5 and 9. The medium of the cultures to be maintained for 6 days was exchanged on day 4, and that of the cultures to be maintained for 9 days on days 4 and 7. The cells were rinsed with CMF-PBS before supplied with fresh medium. After each exchange of medium the T-flasks were gassed again.

The media decanted from the cultures, were centrifuged at 4 000 rev/min for 15 minutes to remove cells and cell fragments and samples from the supernatants were hydrolyzed. The hydrolysates were freed from the interfering chromogens on Dowex 50×8 cation-exchange columns and then used for determination of ha according to the method of ELSON-MORGAN, modified by BOAS [3].

Results

Hexosamine (ha) quantities produced in the presence of gas mixtures of different O_2 concentrations are summarized in Table 1 and Fig. 1; the values are means calculated in the following way: from the value of measurement the own ha content of the medium (77.0 μg/ml) was subtracted and the ha quantity thus obtained was referred to μg cell protein as unit of culture. Deviation from the mean of 5 individual values is indicated by standard deviation (SD).

Table 1 and Fig. 1 demonstrate that with all gas mixtures the highest ha quantity per μg cell protein was obtained after one day of culturing. The highest amount of ha was produced in the presence of 7% oxygen, then at concentrations of 4, 20 and 2%. After 6 days the highest ha production occurred at 4%, followed in decreasing order by 2 and 7%. No ha was produced in the presence of 20% oxygen. After 9 days the cells living in the presence of 7% and 2% O_2 produced small amounts of ha, whereas at a concentration of 4%, the ha production was substantially higher.

Within 24 hours following dispersion and prior to gassing the cells produced minimal amount of ha (0.48 μg ha/$\pm$0.22/μg cell protein).

Investigation of the ha production in different periods of cultivation (3, 6 and 9 days) (Fig. 2) informs us of further details. The production in the first three days is characterized by two measurements (on days 1 and 3). It is striking that in all groups higher ha production was measured after 24 h

Table 1

O_2 content of gas mixture per cent	Duration of cultivation							
	1 day		3 days		6 days		9 days	
	medium I*				medium II*		medium III*	
	μg ha/μg cell protein	SD	μg ha/μg cell protein	SD	μg ha/μg cell protein	SD	μg ha/μg cell protein	SD
20	4.38	$\pm$1.64	1.18	$\pm$1.15	0.00	$\pm$0.44	—	—
7	9.96	$\pm$1.76	3.27	$\pm$1.22	2.50	$\pm$1.45	1.36	$\pm$0.32
4	5.64	$\pm$0.41	3.05	$\pm$0.32	4.81	$\pm$0.71	4.84	$\pm$0.68
2	3.82	$\pm$0.14	3.32	$\pm$0.14	3.94	$\pm$2.11	0.71	$\pm$0.00

* Medium I, II and III were of identical composition; Earle solution containing lactalbumin hydrolysate and yeast extract. SD = standard deviation

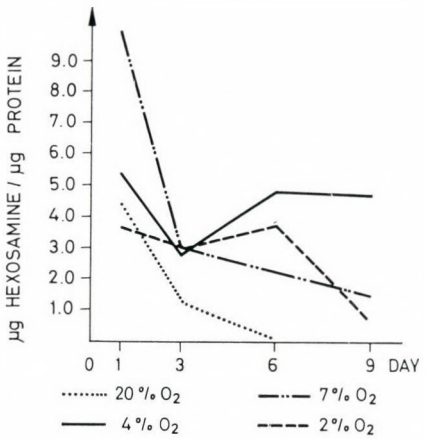

Fig. 1. ha production by fibroblasts from 9-day-old chick embryos in the presence of gas mixtures of different O_2 concentrations

than after 72 h. The difference was 3.20 µg, 6.69 µg, 2.59 µg and 0.50 µg in the presence of 20, 7, 4 and 2% respectively. These differences indicate an actual loss of ha. The sum of the ha production values measured on days 3, 6 and 9 was considered as the total ha production of the cells living in the presence of the corresponding gas mixture. In Table 2 the total ha quantity of the different groups and the percentage distribution of ha production in the culturing periods are summarized. As can be seen in Table 2, the ha

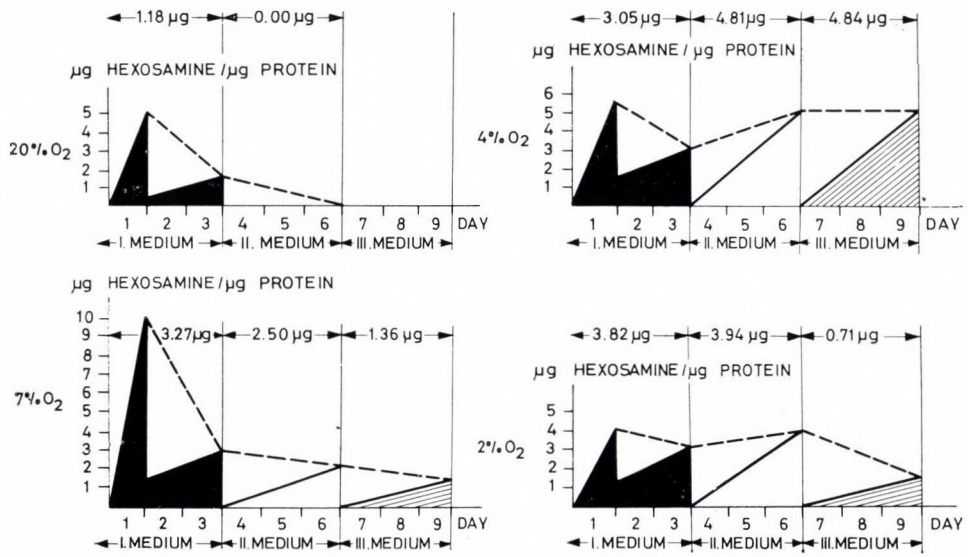

Fig. 2. Change of ha production (———) in different nutritional periods; ha in medium was determined in cultures (for details, see text) 1, 3, 6 and 9-day-old

Table 2

O_2 content of gas mixture per cent	Total ha production (μg ha/μg cell protein)	Distribution of hexosamine production in different periods of culturing (in %)		
		1st period	2nd period	3rd period
20	1.18	100.0	∅	∅
7	7.13	45.8	35.1	19.1
4	12.70	24.0	37.8	38.2
2	7.97	41.3	49.4	9.0

production was the highest in the presence of the gas mixture containing 4% O_2. Under these conditions ha production remained at high level even in the third period of cultivation (see Fig. 3).

Discussion

It has been stated that the majority of connective tissue cells are capable of producing MPS *in vitro* [11, 15, 16]. Under these circumstances, due to an active metabolism [2], various acid MPSs can be produced [2, 13, 16, 25]. Some cells keep their ability for a relatively long time [7, 8, 29], others soon lose it [21, 35].

Numerous authors have dealt with the question how and to what an extent different factors (e.g. pH; glucose or serum content of the medium; addition of glutanime or Co^{++} to the medium etc.) affect the degree of MPS production of certain cells or tissues [9, 28]. One of the factors influencing MPS production is O_2 supply. Numerous studies have been carried out on morphological changes due to an increased or deficient O_2 supply and on the influence of the oxygen concentration on further differentiation of partially determined cells of cartilage or bone origin [1, 20, 32]. BASSETT [1] cultivated cells from the tibia of 20-day-old chick embryos. In the presence of 35% O_2 these cells, owing to "compaction", formed bone tissue, whereas an environment containing 5% O_2 coupled with compaction resulted in cartilage differentiation. HALL [20] cultured membrane bone anlages from 9-day-old chick embryos in the initial stage of ossification. In the presence of 5% O_2 precartilage cells developed in 25% of the cases. Investigating cells isolated from sternal cartilage of 15-day-old chick embryos PAWELEK [32] demonstrated that low concentration of O_2 with simultaneously given thyroxin stimulated chondrogenesis and MPS biosynthesis. Low O_2 concentration without thyroxin administration proved to be ineffective.

The cells studied by us derived from primary fibroblast culture. Their morphological features and other characteristics have been described elsewhere

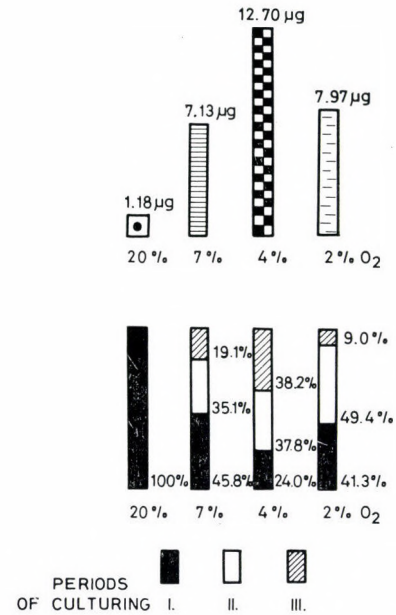

Fig. 3. Total ha production by fibroblasts in the presence of gas mixtures of different O_2 concentrations during 9 days of cultivation. The upper part of the Figure is a comparison of the total ha production in the different groups. The lower part demonstrates the distribution of total ha production (100%) in the different periods of cultivation (see Table 2)

[6, 18, 31]. The gas mixtures containing 7% oxygen proved to correspond to the normal O_2 supply for the cells [6]. Lower concentrations (4% and 2%) proved to bring about a hypoxic, whereas higher concentrations (20%) a hyperoxic, environment. From our results it is apparent that the degree of MPS production is low in hyperoxic milieu and it is somewhat higher at normal and low oxygen supply. On the other hand, in a moderately hypoxic environment marked MPS production was noted. Since the O_2 content of the gas mixture was the only variable in our experiments, it may be concluded that the degree of O_2 supply is capable of modifying the extent of MPS production, and that a hypoxic medium is optimal for the biosynthesis of MPS. Our investigations, however, have failed to give an answer to the question whether it is possible — by changing the O_2 supply — to induce biosynthesis in cells that in general do not produce MPS.

During the period of experimentation the cells preserved their fibroblast shape. No cell forms showing signs of cartilage differentiation were found in our cultures. These findings seem to indicate that a change in the O_2 concentration alone is not sufficient to induce cartilage differentiation of cells. The cells studied by BASSETT [1], HALL [20] and PAWELEK [32] may be regarded as more specialized cells than ours because they originated from older material

and from a special site. Still, further effects ("compaction", thyroxin) were required for their differentiation to cartilage.

More ha was produced after one than after 3 days of cultivation, showing that the rate of ha production is the highest in the earliest period. This finding, which is in agreement with the data of KIMURA [22], is probably due to the abundant substrate (glucose) content of the fresh medium. A decrease in substrate content resulted in a reduced ha production, and the lower values measured on the third day as compared to the 24 hour values seem to indicate that the cells had consumed ha which presumably served as a substrate for cellular metabolism. This supposition is in agreement with that of MCGARRAHAN and MALEY [27], who suggest that ha may be utilized in the processes of the Embden–Meyerhof pathway. We do not know whether loss of ha occurs also in later periods of culturing as we have no experimental data in this respect.

Investigations into the mechanism of effect of oxygen-deficient condition are in progress.

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Acta biol. Acad. Sci. hung., **24**(1—2), 73—81 (1973)

EFFECTS OF THE OXATHIINS DCMO AND DCMOD ON GROWTH, PROTEINS, OXIDIZING ENZYMES, AND HCN PRODUCTION OF A LOW-TEMPERATURE BASIDIOMYCETE

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(Received 1972—09—06)

Abstract

The oxathiins DCMO (carboxin) and DCMOD (oxycarboxin) were found to be fungistatic to the growth of a HCN-producing low-temperature basidiomycete in Petri plate, static liquid and liquid shake cultures. Except for Petri plate cultures, inhibition of growth was temporary. In liquid shake cultures DCMOD was more effective than DCMO in inhibiting mycelial growth and HCN production. Total protein concentrations for the DCMO-treated cultures were similar to controls but were reduced considerably by DCMOD. The disc gel electrophoretic patterns for mycelial proteins and peroxidases were altered by both fungicides, and the numbers of protein bands were reduced as a result of DCMOD treatment. DCMO stimulated peroxidase and particularly polyphenoloxidase activities: whereas, DCMOD decreased the activities of catalase, peroxidase and polyphenoloxidase enzymes.

Introduction

Oxathiin derivatives have been used successfully for controlling plant diseases particularly those caused by fungi belonging to the basidiomycetes. Information on the mode of action of the systemic oxathiin fungicides vitavax (DCMO) and plantvax (DCMOD) is limited. MATHRE [9] found that fungi sensitive to these fungicides absorbed more of the fungicides than resistant fungi. In later work MATHRE [10] reported that the exogenous respiration of *Rhizoctonia solani* was inhibited by DCMO and that of *Ustilago nuda* and *U. maydis* were inhibited by DCMO and DCMOD; however, at low concentrations DCMO stimulated the respiration of the above fungi. MATHRE [10] also found that the incorporation of phenylalanine into protein was only slightly inhibited by DCMO, but the incorporation of uracil into nucleic acid was reduced by 75% and the oxidation of pyruvate and acetate was inhibited. The effects of DCMOD other than for growth and respiration were not reported. The oxathiins are subject to oxidation to a non-fungitoxic form [15], and GROVER and CHOPRA [6] cultured isolates of *R. solani* and *R. batati cola* on otherwise lethal concentrations of DCMO and DCMOD. The effects of these oxathiins on pro-

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teins and oxidative enzymes of ageing fungus mycelium have not been reported. The objectives of this report were to show the effects of DCMO and DCMOD on mycelial growth, proteins, and oxidative enzymes catalase, peroxidase, and polyphenoloxidase, and HCN production of the HCN-producing low-temperature basidiomycete since these parameters for ageing cultures of this fungus have already been dealt with in some detail [14].

Material and method

The fungus used for this study was a HCN-producing low-temperature basidiomycete, isolate W_2, grouped under type B cultures by WARD and co-workers [19]. Stock cultures of this isolate were maintained in test tubes on a synthetic-agar medium [17] at 3—5 °C.

Growth studies: radial growth and weight of mycelium produced in the presence of the fungicides 2,3-dihydro-5-carboxanilido-6-methyl-1, 4-oxathiin (DCMO) and 2,3-dihydro-5-carboxanilido-6-methyl-1, 4-oxathiin-4,4-dioxide (DCMOD), were determined at 15 °C for Petri plate, static liquid, and liquid shake cultures. Concentrated solutions of DCMO and DCMOD were prepared by dissolving each fungicide in 95% ethanol to give a final concentration of 2.0×10^{-4} mol/l. The ED_{50} of each fungicide was prepared by diluting the concentrated solutions with 20% ethanol, after which the solutions were passed through 0.45 μ Millipore filters. One ml aliquot of the sterile diluted solutions was added to 50 ml of sterilized basal synthetic medium at 45—50 °C prior to inoculation. Inoculation and preparation of the synthetic agar medium were essentially the same as described by WARD and COLOTELO [17] and SEKHON and COLOTELO [14]. Controls consisted of the above medium with one ml aliquot of sterilized 20 per cent ethanol without fungicide. Growth (diam. of colony in mm) on agar medium in Petri plates was measured at two-day intervals for a total period of 24 days. Yields of mycelia (dry wt., mg/flask) from liquid static cultures were obtained 16 and 24 days after inoculation, and from liquid shake cultures 12, 16, and 24 days after inoculation. The procedures for harvesting and dry weight determinations were described previously by SEKHON and COLOTELO [14].

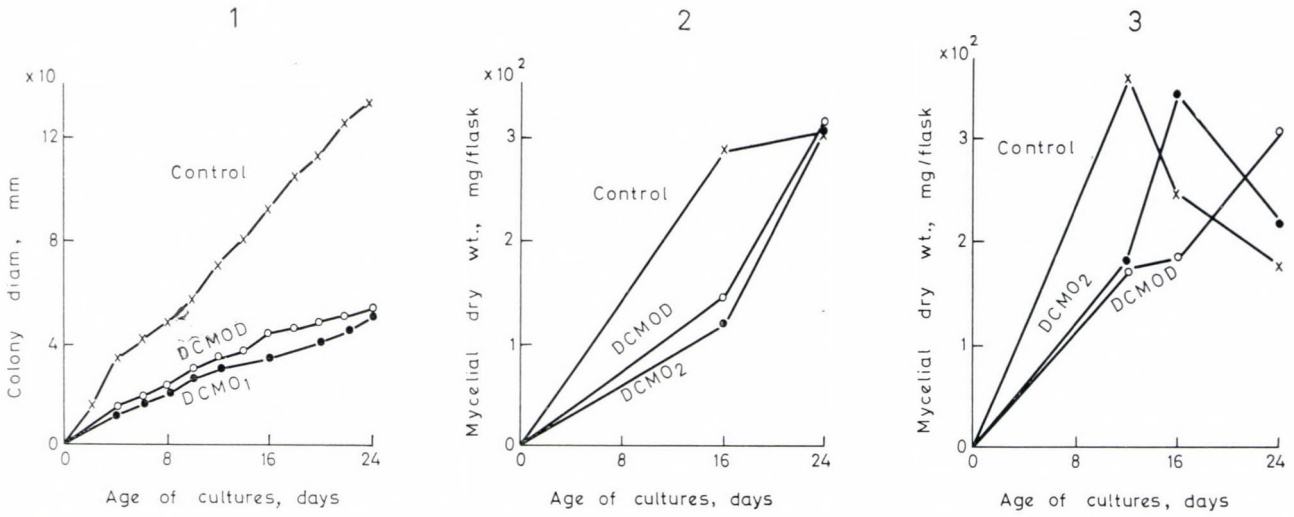
Protein determinations: Approximately 5 g of mycelium (fresh wt.) from each harvest was used for the extraction of soluble proteins for electrophoretic studies. These procedures were described by SEKHON and COLOTELO [14]. Total protein content was measured using the procedure of LOWRY and co-workers [8]. Disc electrophoretic separation and detection of proteins were carried out following the procedures of DAVIS [2]. Peroxidases in the gels were detected with benzidine dihydrocholoride reagent (Bulletin, Special Subject, Enzyme analysis, Canal Industrial Corporation, Rockville, Maryland).

Enzyme activities: Catalase activity was determined using the method of BEERS and SIZER [1]. The breakdown of H_2O_2 was followed spectrophotometrically at 240 nm and the decrease in absorbance at 10 sec intervals was recorded. Peroxidase activity was determined by measuring the rate of colour development at 460 nm with the decomposition of H_2O_2 using o-dianisidine as the hydrogen donor as described in the manual "Enzymes, Enzyme reagents" (Worthington Biochemical Corporation, Freehold, N. J.). Polyphenoloxidase activity was determined by measuring the increased absorbance at 420 nm as the result of ferricyanide production in the presence of catechol using the procedure of HALL and co-workers [7]. For peroxidase and polyphenol oxidase activities the increase in absorbance of the reaction mixtures was recorded at 10 sec intervals for 1—2 min. Enzyme activities are expressed as units per mg of soluble fungal protein.

HCN: HCN release in 12-, 16-, and 24-day old liquid shake cultures, with and without fungicide, was determined using the method of WARD and LEBEAU [18]. The HCN collected at 12, 16, and 24 days from the day of inoculation was the accumulated total to that date. All experiments were repeated once and each value expressed is the average of six determinations.

Results and discussion

Growth studies. The ED_{50} values for DCMO and DCMOD using agar medium were 0.5×10^{-5} mol/l and 10×10^{-5} mol/l respectively (Fig. 1). In static liquid cultures (Fig. 2) the yields of mycelium were approximately one-half



Figs 1—3. Effects of oxathiins DCMO and DCMOD on the growth of a HCN-producing low-temperature basidiomycete grown on a synthetic medium at 15 °C. *Fig. 1.* Petri plate cultures.

Fig. 2. Static liquid cultures. *Fig. 3.* Liquid shake cultures. Concentration of fungicides at time of inoculation; DCMO[1], 0.5×10^{-5} mol/l; DCMO[2], 0.25×10^{-5} mol/l; DCMOD, 10×10^{-5} mol/l (Concentrations of fungicides predetermined to give a 50 per cent reduction in mycelial yield when maximum or near maximum reached in controls)

that of controls at 16 days when concentrations of DCMO and DCMOD were 0.25×10^{-5} and 10×10^{-5} mol/l respectively at the time of inoculation; however, at 24 days the yields of mycelium were slightly higher than for the controls. Maximum yield of mycelium in untreated liquid shake cultures were obtained 12 days following inoculation and the ED_{50} values of the fungicide-treated cultures were similar to the static liquid cultures. Maximum yield of mycelium from DCMO-treated liquid shake cultures were obtained 16 days after inoculation and this yield was comparable to that of 12-day-old control cultures; and as a result of DCMOD treatment a high yield of mycelium was not obtained until 24 days at which time the experiment was terminated. These studies show that DCMO was more effective at lower concentrations than DCMOD in inhibiting the growth of this low-temperature basidiomycete. These findings are in part supported by the reports for other fungi by EDGINGTON and BARRON [3], MATHRE [9], SNEL and EDGINGTON [15], and EDGINGTON and coworkers [5] who found that fungistasis by DCMO occurred at a lower concentration than for DCMOD. These studies show that the method of culturing should be taken into consideration when evaluating and interpreting the effect of fungicides, and the data for static liquid and liquid shake cultures indicate that either the fungus became adaptive to these fungicides or the fungicides were degraded. Adaptation to DCMO and DCMOD by *R. solani* and *R. bataticola* was shown by GROVER and CHOPRA [6]. The virulence of these isolates was reduced; however, the cultures grown on DCMOD regained their virulence but those grown on DCMO did not. They suggested that a possible mutation in pathogenicity occurred when cultures were grown on DCMO and that DCMOD was involved only in temporary enzymatic change.

Total protein. In control cultures there was a concomitant decrease in protein (Table 1) with a decrease in yield of mycelium (Fig. 3) in ageing cultures. Although the yields of mycelia for DCMO- and DCMOD-treated cultures at 12 days were similar (Fig. 3), being 50% of controls, the protein concentration of the DCMO-treated cultures were slightly higher than controls but nearly seven times greater than that observed for the DCMOD-treated cultures. At 16 days the protein concentration of the DCMO-treated cultures had decreased even though the yield of mycelium had increased to a value which was almost that noted for 12-day-old control cultures. In the 16-day-old DCMOD-treated cultures there was a 50% increase in protein with only a slight increase in mycelial yield over that of 12-day-old cultures. Data presented in this paper indicate that DCMO did not inhibit protein synthesis of the low-temperature basidiomycete even though mycelial growth was inhibited. For *R. solani*, MATHRE [10] noted a decrease in protein synthesis and inhibition of hyphal extension in Petri plate cultures containing DCMO. However, GROVER and CHOPRA [6] reported that *R. solani* and *R. bataticola* became adapted to grow in media containing lethal concentrations of DCMO

Table 1

Effects of oxathiins DCMO (0.25×10^{-5} mol/l) and DCMOD (10×10^{-5} mol/l) on total proteins and activities of catalase, peroxidase, and polyphenoloxidase enzymes of a HCN-producing low-temperature basidiomycete, isolate W_2, grown in liquid shake cultures on a synthetic medium at 15 °C*

Treatment	Age of culture (days)	Total protein (mg/g dry wt. of mycelium)	Enzyme activity (units per mg protein)		
			Catalase	Peroxidase	Polyphenol-oxidase
Control	12	58.4	740	56	5.79
	16	40.4	650	54	4.22
Change due to increasing age of cultures		—18.0	—90	— 2	— 1.57
DCMO	12	61.8	600	52	19.49
	16	46.8	470	94	4.08
Change due to increasing age of cultures		— 15.0	— 130	+ 42	— 15.41
DCMOD	12	8.9	50	3	0.59
	16	12.9	30	12	0.49
Change due to increasing age of cultures		+ 4.0	—20	+ 9	— 0.10

* Concentrations of fungicides predetermined to give a 50 per cent reduction in mycel ia yield when maximum or near maximum reached in 12-day-old control cultures

and DCMOD and that the adapted isolates differed morphologically from the parent strains.

Electrophoresis of proteins and peroxidases. The numbers of protein bands observed for 12-day-old control, DCMO-, and DCMOD-treated cultures were 25, 25, and 20, respectively. With increasing age of cultures, i.e., at 16 days, the numbers of bands for control cultures decreased to 20 and for the DCMO-treated cultures remained at 25, while there were only 15 bands for the DCMOD-treated cultures. As well as numbers of bands, the protein band patterns for the DCMO and DCMOD-treated cultures differed from one another and these differed from those of the controls.

Four and two peroxidase bands were observed for 12- and 16- day-old control cultures, respectively; whereas, in the 12-day-old DCMO-treated cultures 11 bands appeared, and at 16 days only five bands were noted. There were eight and six bands from 12- and 16-day-old DCMOD-treated cultures respectively. Wide diffuse bands were observed, particularly from 16-day-old control and DCMO-treated cultures, but such bands were not present from cultures treated with DCMOD.

Enzyme activities. Catalase activities for control and fungicide-treated cultures decreased with increasing age of cultures (Table 2). At 12 days, catalase activities for the DCMO- and DCMOD-treated cultures were approximately 80 to eight %, respectively, of those obtained for control cultures;

Table 2

Effects of oxathiins DCMO (0.25×10^{-5} mol/l) and DCMOD (10×10^{-5} mol/l) on HCN production by a HCN-producing low-temperature basidiomycete, isolate W_2, grown on a synthetic medium in shake culture at 15 °C*

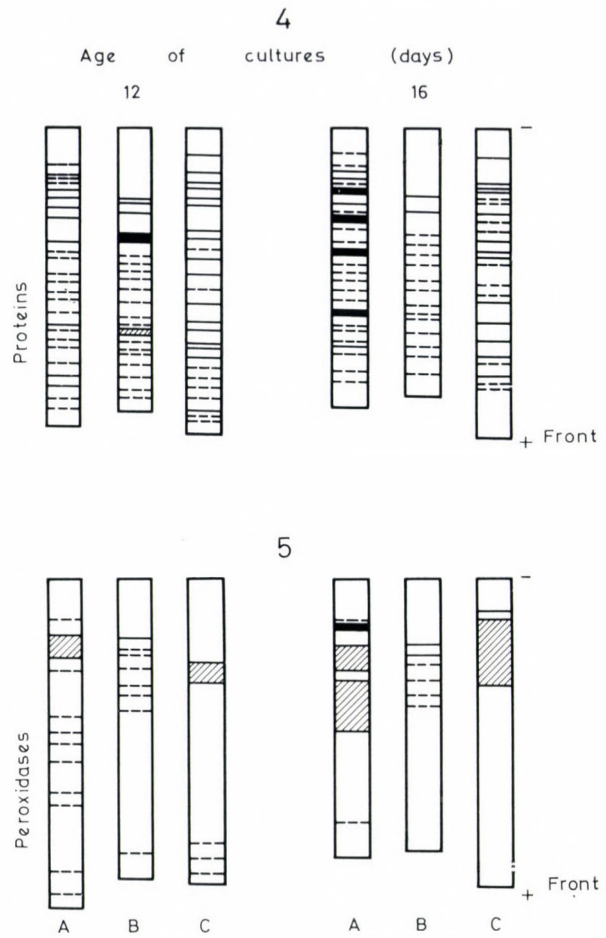
Treatment	HCN (parts/10^6/flask)		
	Age of cultures (days)		
	12	16	24
Control	—	1140	2810
DCMO	—	40	2460
DCMOD	—	—	79

* Concentrations of fungicides predetermined to give a 50 per cent reduction in mycelial yield when maximum or near maximum reached in controls

and at 16 days, the activities were approximately 72 and five %, respectively, that of controls. The decreases in catalase activities for ageing control and DCMO-treated cultures were associated with decrease in protein. However, in the DCMOD-treated cultures there was a 40% decrease in activity although there was a 50% increase in protein content. In the control cultures activity decreased with a decrease in mycelial dry weight; whereas, in the fungicide-treated cultures the decrease occurred when the mycelial dry weights were increasing.

Peroxidase activities of 12- and 16-day-old control cultures were very similar (Table 2). Peroxidase activity of the DCMO-treated cultures at 12 days was similar to control cultures but in the DCMOD-treated cultures the activity was approximately five per cent that of controls. At 16 days, the peroxidase activity of the DCMO cultures had increased and was approximately 40% higher than controls. In the DCMOD-treated cultures at 16 days, peroxidase activity was four times that at 12 days but still about one-quarter that of controls and one-seventh that of 16-day DCMO-treated cultures.

The trends for polyphenoloxidase activities were similar to that of catalase (Table 2) in that activities for the control and fungicide-treated cultures were higher at 12 days than at 16 days. The highest activity was recorded for DCMO-treated cultures at 12 days, and at this time this activity was 3.5 and 33 times higher than control and DCMOD-treated cultures, respectively. At 16 days the activities for control and DCMO-treated cultures were quite similar. The activities for the DCMOD-treated cultures were approximately one-tenth that of control cultures at 12 and 16 days. The adaptation of the low-temperature basidiomycete to DCMO and DCMOD may in part be due to degradation of the chemicals by the fungal oxidizing enzymes as indicated by the increased peroxidase and polyphenoloxidase activities (Table 1), partic-



Figs 4—5. Effects of oxathiins DCMO (0.25×10^{-5} mol/l) and DCMOD (10×10^{-5} mol/l) on gel electrophoretic patterns of proteins and peroxidases of a HCN-producing low-temperature basidiomycete, W_2, grown in shake culture on a synthetic medium at 15 °C. A = DCMO; B = DCMOD; C = control (Concentrations of fungicides predetermined to give a 50 per cent reduction in mycelial yield when maximum or near maximum reached in controls)

ularly in the DCMO-treatment. MATHRE [10] reported that the inhibition of respiration and metabolism of acetate in *R. solani* and teleospores of *Ustilago nuda* were greatly reduced when oxidized analogs and compounds structurally related to DCMO were used, and that the toxicity of DCMO was greatly reduced when the sulphur atom was oxidized. In bean roots, DCMO, in contrast to DCMOD, was found to be more readily oxidized to a non-fungitoxic form [16].

HCN. HCN production which increased with age of cultures was retarded by DCMO- and DCMOD-treatments (Table 2). In control cultures HCN was observed only after a decrease in mycelial yield had occurred, and in the DCMO-treated cultures HCN production was first noted at 16 days and was greatest after there was a decrease in mycelial yield. In the DCMOD-treated cultures HCN production was first observed at 24 days, and the mycelial yield at this time was slightly lower than that of 16-day DCMO-treated cultures. WARD and LEBEAU [18] and SEKHON and COLOTELO [13, 14] reported that HCN was associated with autolysis although WARD and THORN [20] found that HCN was produced throughout the growth of this fungus, particularly when glycine was substituted for aspargine as the nitrogen source.

Acknowledgement

Financial assistance from the Alberta Agricultural Research Trust is gratefully acknowledged.

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Acta biol. Acad. Sci. hung., **24**(1—2), 83—90 (1973)

ELECTRON MICROSCOPIC DEMONSTRATION OF ENERGY PRODUCTION IN MOLLUSCAN NEURONS

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(Received 1972—09—07)

Abstract

Energy-dependent accumulation of Sr^{2+} was used for the *in situ* electron microscopic visualization of mitochondrial energy production of molluscan neurons. Strongest precipitation was observed using succinate as respiratory substrate, whereas α-ketoglutarate, malate or pyruvate resulted in a weaker reaction only slightly different in intensity from that supported by endogenous substrates. Successful inhibition by 0.5 mmol/l 2,4-dinitrophenol and 2—4 mmol/l KCN proved the participation of the respiratory chain and energy coupling mechanism in the reaction. Besides mitochondria, cytosomes (yellow pigment granules) displayed an ability to accumulate Sr^{2+} showing that in the molluscan neurons energy-linked functions are proceeding both in the mitochondria and cytosomes. The latter structures are suggested to be derived from mitochondria.

Introduction

Previous studies on molluscan neurons showed that not only the mitochondria but also the yellow-pigmented granules, called cytosomes by NOLTE and co-workers [9], contain succinate dehydrogenase and cytochrome oxidase [12, 16]. Based on this histochemical and other morphological observations the mitochondrial origin of cytosomes suggested earlier by others [3, 8] was supported [13]. It seemed, therefore, to be of considerable interest to decide, whether the cytosomes possess also an energy-coupling mechanism characteristic for mitochondria.

For the visualization of energy production at the ultrastructural level massive, respiration-dependent accumulation of Sr^{2+} may be utilized. It was shown, namely, by GREENAWALT and CARAFOLI [4] that at the expense of energy produced during respiration, isolated mitochondria are able to accumulate strontium and phosphate in such quantities that they become precipitated as electron dense deposits within mitochondria.

Material and method

Cerebral, visceral and pedal ganglia of adult (14—18 cm long) specimen of *Anodonta cygnea* were used. The ganglia were incubated in a medium described by KERPEL-FRONIUS and HAJÓS [7] except that NaCl was omitted. The decrease of osmolarity was necessary, as the tissues of *A. cygnea* are isotonic with the hemolymph displaying only 0.04 osmol pressure [10]. The mixture was composed from stock solutions as follows;

1 ml 0.1 mol/l Tris-HCl buffer, pH 7.0;
2 ml distilled water;
0.5 ml 0.1 mol/l succinate, pyruvate, malate or α-ketoglutarate adjusted to pH 7.0 with NaOH buffered at pH 7.0 by NaOH;
1 ml 0.02 mol/l $Sr(NO_3)_2$;
0.5 ml 0.04 mol/l K-Na-phosphate buffer, pH 7.0.

The incubation was carried out for 45 minutes at room temperature in open vessels. The incubation was followed by fixation in 2% OsO_4, buffered with s-collidine at pH 7.2 [2], for 30 min at 0° C and for 10 min at room temperature. Following dehydration in ethanol the blocks were embedded in Durcupan ACM (Fluka). Sections were cut by an LKB Ultrotome III and were stained with lead citrate [11].

The specificity of the reaction was controlled either by omitting the respiratory substrate or by inhibiting the respiratory chain and energy coupling mechanism with 2—4 mmol/l KCN and 0.5 mmol/l 2,4-dinitrophenol (DNP), respectively.

Results and discussion

The reaction is confined to a narrow surface layer of the blocks due to limited penetration of the medium. Therefore the results were evaluated in this zone, which was selected as described by KERPEL-FRONIUS and HAJÓS [6].

The incubation without prefixation did not induce any serious structural damage. All the subcellular elements remained recognizable, although the mitochondria loaded with precipitate showed considerable swelling (Figs 1, 3). In the layers without reaction or after inhibition of Sr^{2+} uptake the mitochondrial swelling was less pronounced similar to the observation made on mammalian tissues [7].

Using succinate as respiratory substrate an intense Sr^{2+} accumulation was observed in the form of large, dense granules localized in the inner compartment of the swollen mitochondria (Fig. 1). Also on the internal membranes of some cytosomes a finely granular precipitate of high electron density appeared (Fig. 2), never seen in them under normal circumstances. However, the majority of cytosomes remained free of reaction even in the surface layer where otherwise the mitochondria showed intense precipitation. It should be noted, furthermore, that needle-like reaction was observed neither in the mitochondria nor in the cytosomes. According to GREENAWALT and CARAFOLI [4], the structural form of the Sr^{2+}-phosphate deposits may be determined by the rate of accumulation insofar as granular precipitation indicates a less intense reaction than a needle-like reaction product. Thus succinate-supported Sr^{2+} uptake seems to be less efficient in the nervous tissue of *A. cygnea* than in mammalian tissues [5, 7].

Complete inhibition of Sr^{2+} accumulation by DNP (Fig. 4) and a significant decrease of precipitation following KCN treatment prove that the reaction is dependent on the function of the respiratory chain and energy coupling mechanism both in the mitochondria and cytosomes. In blocks incubated in a medium containing no respiratory substrate the mitochondria and some cytosomes showed Sr^{2+} accumulation, however, much less than in the presence

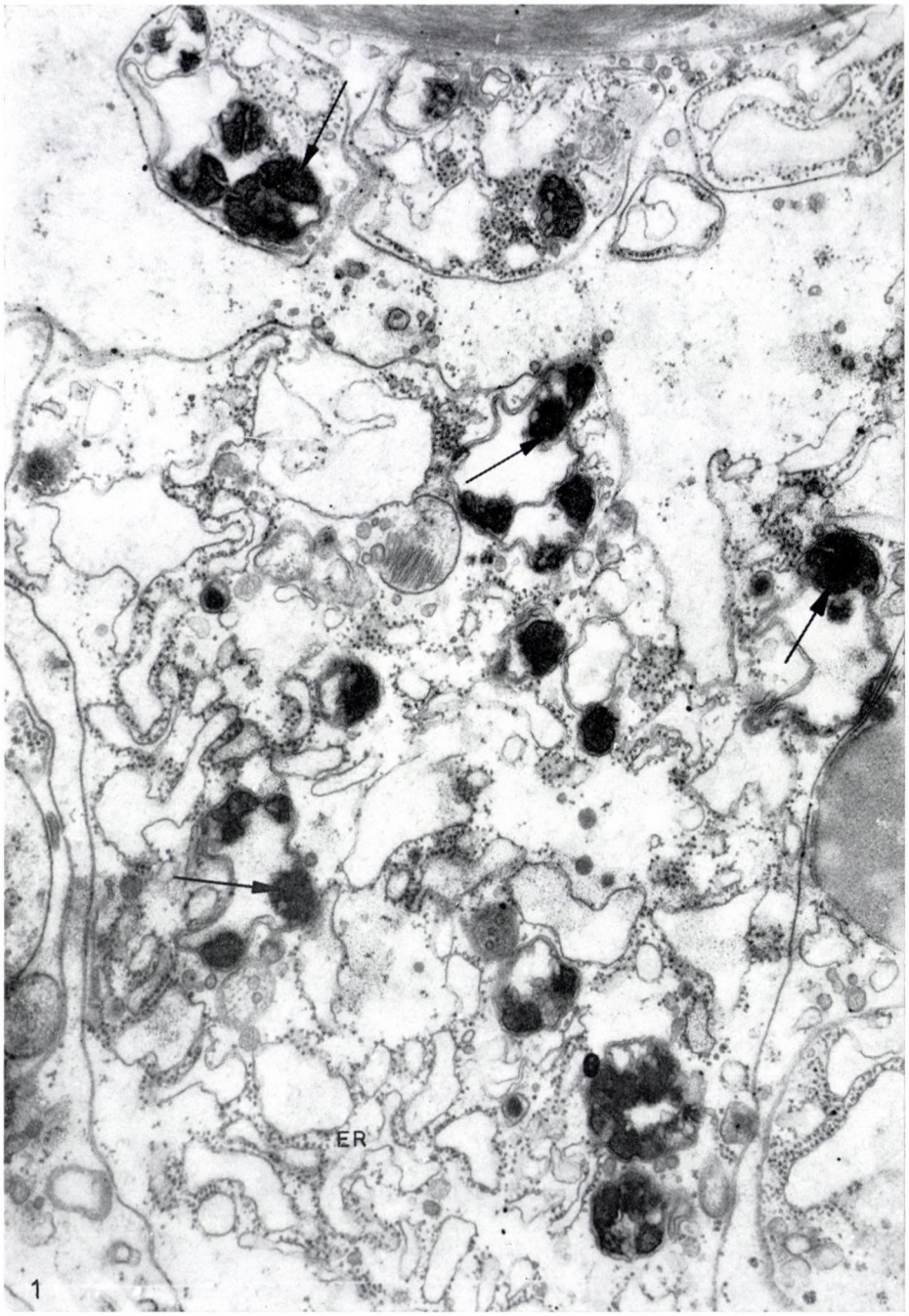

Fig. 1. Succinate-supported Sr^{2+}-accumulation in the mitochondria of a nerve cell in the visceral ganglion. Arrows indicate the Sr-phosphate deposits of high electron density. ER = endoplasmic reticulum; × 25 000

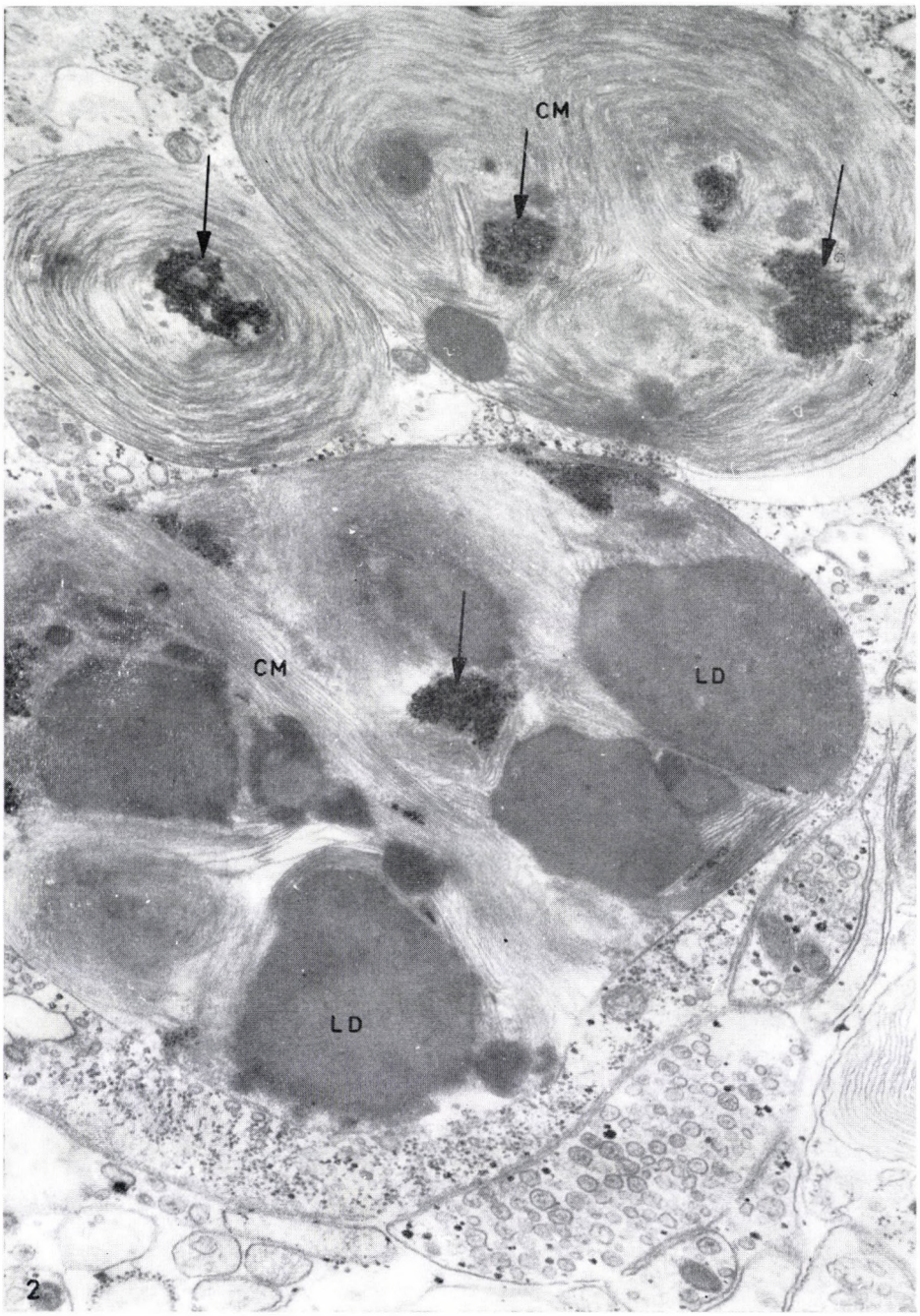

Fig. 2. Succinate supported Sr^{2+} accumulation in the cytosomes of a nerve cell in the cerebral ganglion. Arrows indicate the finely granular Sr-phosphate deposits within the cytosomes. LD = lipid droplets of the cytosome; CM = cytosomal internal membranes; × 25 000

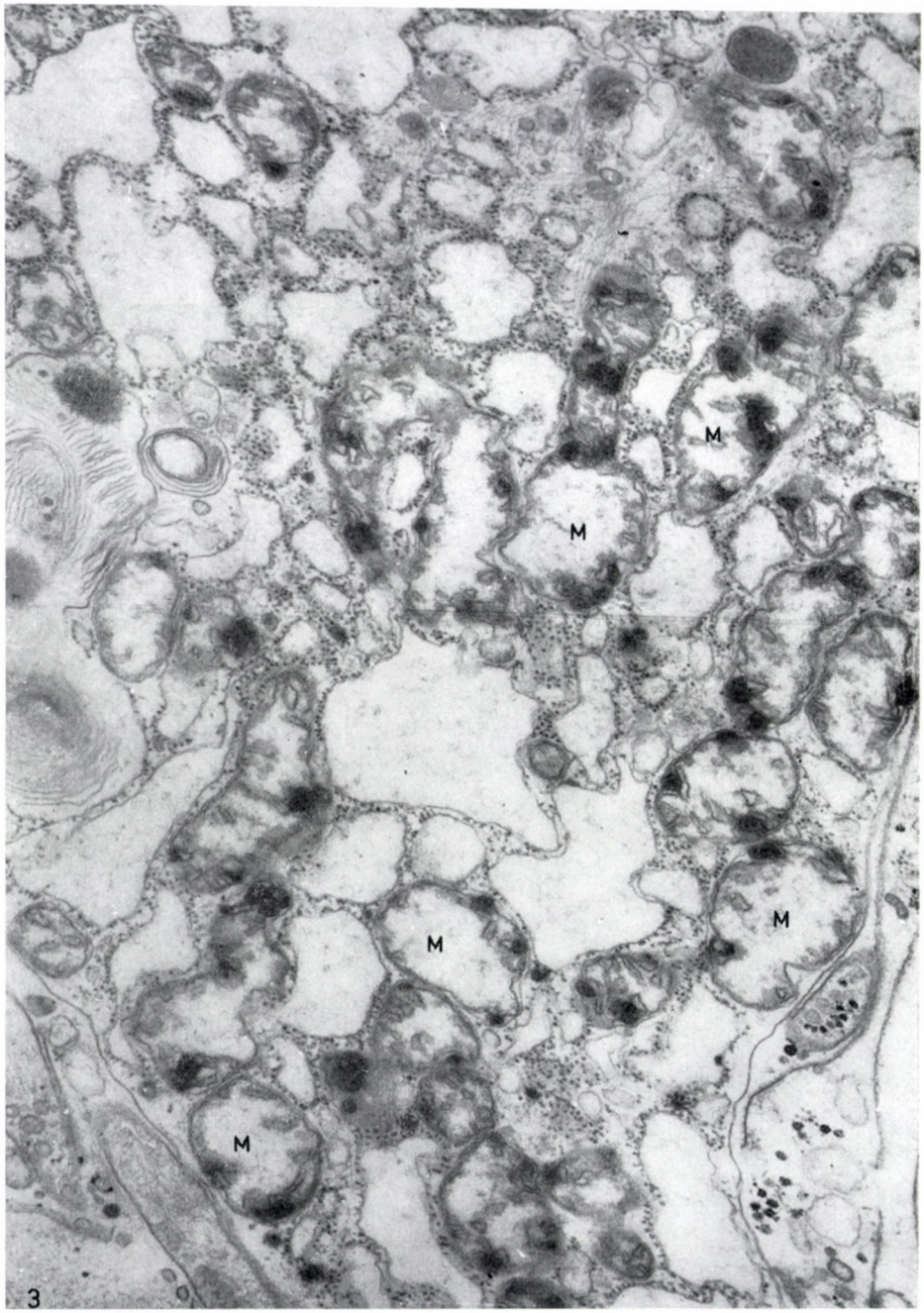

Fig. 3. Sr^{2+}-accumulation in the mitochondria (M) of the cerebral ganglion, supported by endogenous substrates. The reaction is of much lower intensity than shown in Fig. 1; ×25 000

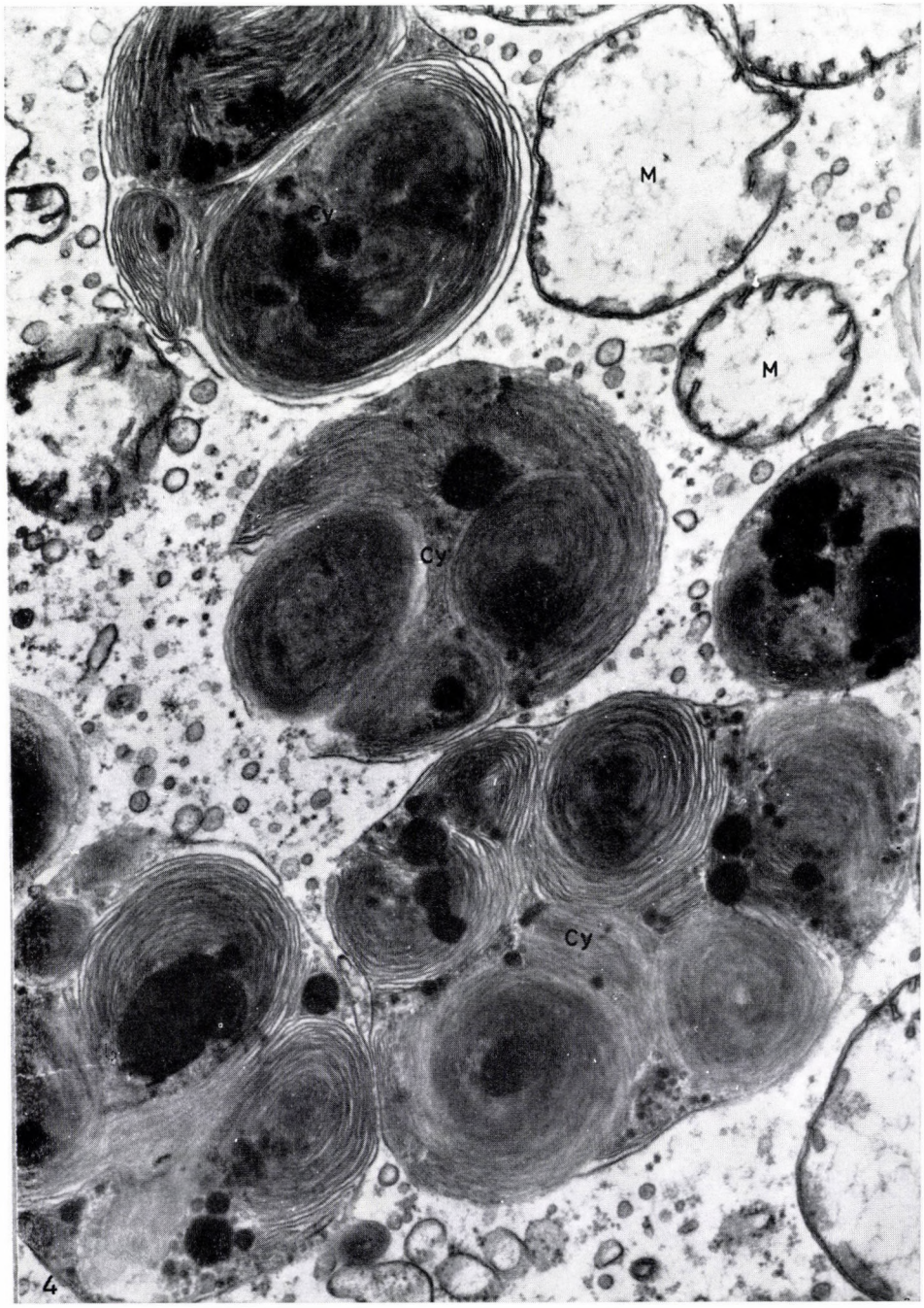

Fig. 4. Inhibition of Sr^{2+}-accumulation by 0.5 mmol/l 2,4-dinitrophenol in the cerebral ganglion. Note the absence of Sr-phosphate deposits both in mitochondria (M) and cytosomes (Cy). ×27 000

of succinate. This reaction may be due to endogenous substrates, since it was as sensitive to DNP and KCN as succinate-supported Sr^{2+} accumulation. This assumption seems to be justified, since considerable amount (0.05—0.4 per cent) of succinic acid has been found in some bivalves [1].

The other respiratory substrates used (pyruvate, α-ketoglutarate and malate) resulted in a weaker reaction than succinate. It was found to be difficult to differentiate the reaction from that supported by endogenous substrates because of the low reaction intensity. Therefore it is impossible to draw any conclusion regarding the presence of the respective primary dehydrogenases catalysing the first step of substrate oxidation.

The demonstration of succinate-supported Sr^{2+} accumulation in the cytosomes corroborate earlier findings showing the presence of succinate dehydrogenase and cytochrome oxidase in these organelles [12, 16]. Succinate dehydrogenase together with a functional electron transport chain and energy coupling mechanism are considered to be characteristic of mitochondria. These findings thus definitely prove the mitochondrial origin of cytosomes as suggested earlier [3, 8, 13, 16]. The functional significance of cytosomal energy production is not yet clear. As is discussed elsewhere, succinate-supported Sr^{2+} accumulation becomes highly activated in the cytosomes parallel with their breakdown during prolonged anoxia [14, 17]. Most probably a lipochrome pigment is used as a final electron acceptor under these conditions [15, 18, 19]. It seems probable, therefore, that cytosomes play an essential metabolic role only under anaerobic conditions which are tolerated for a remarkable time by numerous Molluscs possessing a large number of cytosomes.

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Acta biol. Acad. Sci. hung., **24**(1—2), 91—94 (1973)

TESTING OF A HUNGARIAN OSTEOPLASTIC POLYAMID (METAMID) ON LIVING CELL CULTURES

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(Received 1972—09—15)

Abstract

Authors investigated the cell-damaging effect of a Hungarian polyamid "Metamid" on HeLa cell cultures. Metamid did not alter the reproduction rate of the cells. The cells displayed the same adherence and proliferation as well as nearly identical mitotic index on the Metamid plates as the control cultures on glass slides.

Introduction

In the literature of bone surgery problems of arthroplasty and osteoplasty, especially those concerning the correction of the hip joint and the "internal prostheses" have been most thoroughly discussed in recent years. Because of the advantageous properties of newly-created plastics (high solidity, high coefficient of elasticity, good wear resistance and last but not least, the tissue compatibility) metal-plastic combinations are more and more favoured in contrast to the pure metallic prostheses.

The present paper analyzes the effect on tissue cultures of a polyamid (Metamid) made in Hungary and issued by Metalloglobus. Similar investigations have scarcely been reported in the literature [1, 2, 4].

According to our observations, [1] cell cultures, especially those on slides, are suitable for testing the toxicity of plastics [2]. Metamid did not alter the reproduction rate of cells, thus its cell-damaging effect can be excluded with high probability.

Material and method

HeLa cell cultures were used. The composition of the growth medium; 85% Parker TC 199 solution, 10% bovine serum, 5% lactalbumin [3]. Cells grown in Roux-flasks of 500 ml were separated from the glass wall using 0.02 % Versene, washed in saline and suspended in fresh medium, 250 000—300 000 cells per ml. This suspension was distributed in test tubes 3 ml in each. There was a cover slip of 0.5×1.0 in surface in each tube. A half of them was made of glass compatible for cell growth, the other half, 0.5 mm in thickness, was made of Metamid. The cover slips were sterilized and carefully cleaned before use.

The tubes were tightly rubber-stopped. No artificial gas mixture was used. The tubes were incubated at 37 °C.

After the first 48 h, 2 tubes of each group were opened by every 24 h. The cover slips

were placed in a 3 : 1 mixture of ethanol–glacial acetic acid for 1 h, then stained with 0.1% toluidin blue dissolved in veronal buffer at pH 4.

The stained cover slips were mounted in colourless gum-arabic and examined in light microscope using a magnification of ×800.

The mitotic index was determined on the 3rd and 5th days by counting 3 000 cells in each culture. The experiment was closed on the 5th day.

Results

After 2 days of culturing large, sometimes even confluent colonies of cells were seen on both the Metamid and glass cover slips (Fig. 1a). The plastic slides were less translucent than the glass ones and even their surface was

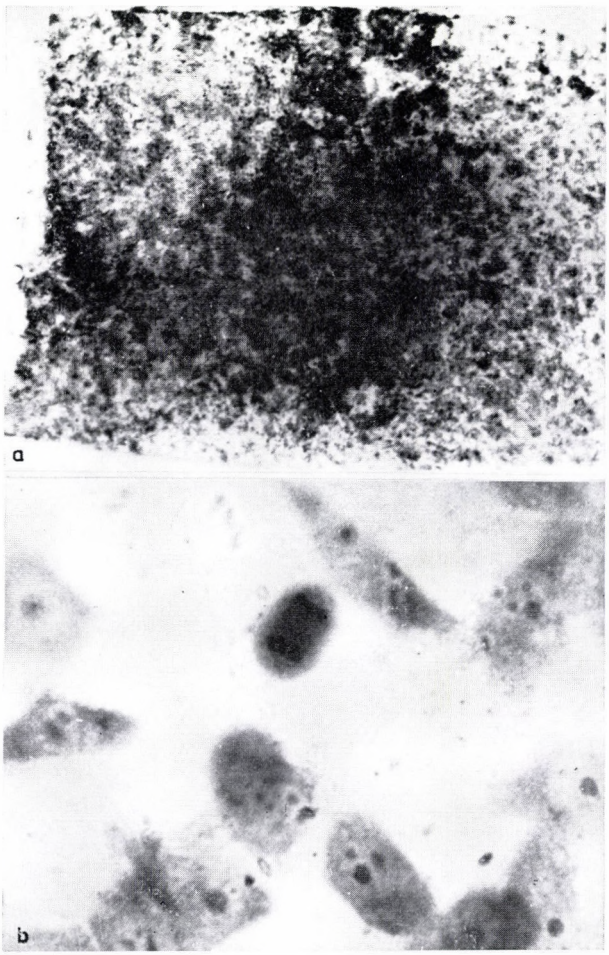

Fig. 1. HeLa cell culture of 3 days on Metamid plastic slide. (a) = Polished plastic slide in full size. More or less contacting cell colonies on the surface of the plastic slide. Toluidin blue staining: ×100. (b) The same as on the Fig. 1a. in centre a dividing cell between cells in interphase. ×800

somewhat uneven, therefore the cells could not be examined under immersion objective. The picture was satisfactorily clear, when a dry objective of ×40 magnification (total magnification ×800) was used and the mitotic forms were well distinguishable (Fig. 1b). On the basis of the mitotic index from the Metamid cover slips were as suitable for culturing of HeLa cells as the glass.

Mitotic index
(Dividing cells per 1 000 cells)

	3rd day	5th day
Control	27 ± 2	19
Metamid	26 ± 2	20

Discussion

Established cell cultures, representing easily treatable living cells, are widely used even apart from the virus culturing. Such cells are sensitive to the alteration of the environment and the culturing medium. From the point of wiew of their life cycles even the solid substrate to which they attach is of great significance. If the culturing medium or the solid substrate is unsatisfactory or of toxic effect, the cells do not attach to the surface and the culture dies within several hours.

The advantageous indicative character of cell cultures has already been used for testing cell-damaging effect of plastics: powerized plastics were placed into the cultures and the form and grows of attached cells were investigated under the phase contrast microscope.

We prepared Metamid thin slides, which are translucent and thus suitable for light microscopy. The slides were placed in cell suspension so that the suspended cells sedimented on, and attached to the slides. This type of preparation allowed, in contrast to the former ones, to analyze not only the morphology and form of colonies and cells grown on the slides, but also to determine the mitotic index, a sensitive indicator of cell multiplication.

The HeLa cells attached to the surface of Metamid and proliferated there. This indicates that no cell-damaging substance diffused into the medium in a toxic concentration from this type of plastics. The attachment of the cells and reproduction rate identical with that of controls involves furthermore that the surface of Metamid is indifferent for living cells, they can get easily in contact with that without any pathological alteration of their metabolism.

Thus, Metamid displayed no observable cell-damaging effect, therefore there can be no objection against its grafting in the human organism, at least from this respect.

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Acta biol. Acad. Sci. hung., **24**(1—2), 95—101 (1973)

SOME CHARACTERISTICS AND RENNETIC ACTIVITY OF THE PURE RENNIN-LIKE ENZYME FROM PENICILLIUM CITRINUM

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(Received 1972—09—21)

Abstract

No time-lag was required for the formation of the milk clot by the pure rennin-like enzyme of *Penicillium citrinum* 805. High substrate concentrations seemed to affect the rate of the enzymic stage of rennin action and hence clotting of milk was retarded. The enzyme action was optimal at 62 °C and at pH 6.1. Under these conditions, the enzyme clotted 4 000 times its own weight of skimmed milk within 30 s. The stability of the enzyme was maximal at pH 5.89 in the presence of the substrate, the enzyme was more stable at pH values above than below the optimum. The milk-clotting activity was diminished by oxidizing agents and was unaffected by reducing agents. No reactive sulfhydryl groups seeemed to be present in the active site of the enzyme. Urea as well as some animal sera completely blocked the enzyme action. The enzyme contains 17 amino acid residues.

Introduction

In previous papers [1, 2], the distribution pattern of milk-clotting and proteolytic activities in some fungi as well as the preparation of a milk-clotting enzyme from *Penicillium citrinum* have been reported. In this respect, the partially purified enzyme of *P. citrinum* exhibited high milk-clotting and low proteolytic activity. Accordingly it was considered as a rennin-like enzyme. The present work was undertaken to investigate some characteristics of the pure extracellular rennin-like enzyme preparation of *Penicillium citrinum* (PRLE). These studies included the effect of urea and some animal sera on the milk-clotting activity. Cheeseman [4] found that calf rennin was rapidly inactivated in solutions containing urea. On the other hand, animal sera were shown by many authors [6, 8, 11, 12] to inhibit clotting of milk by rennin. Available knowledge about the effect of such agents on the activity of microbial rennin enzymes is, however, still scanty. This study may give useful information on the rennin-like enzyme of *Penicillium citrinum* and hence leads to its better evaluation and utilization.

Material and method

Organism. Penicillium citrinum 805 (NRRL) was used throughout. Cultivation was done as described by Abdel-Fattah and co-workers [1, 2].

Culture medium. This was composed of corn steep matter (2% mass/vol.) and lactose (2% mass/vol.).

Preparation of rennin-like enzyme. After incubation for 7 days at 30 °C, the culture filtrate was precipitated with 2 volumes of ice-cold acetone. After drying overnight over P_2O_5, the precipitate was dissolved in distilled water and cooled. This enzyme solution was then fractionally precipitated with ice-cold acetone. The fraction precipitated at 50% (v/v) acetone was used as "rennin-like enzyme".

Electrophoresis of rennin-like enzyme preparation. Paper electrophoresis was done with the Elphor apparatus and 0.02 mol/l acetate buffer (pH 3.42): 300 V (0.2 mA) strip) was applied at room temperature.

Measurement of milk-clotting activity. This was done according to the method of BERRIDGE [3]: unless otherwise specified, a 2.5 ml volume of the buffered (0.02 mol/l acetone buffer, pH 4.0) enzyme sample was incubated with 10 ml reconstituted skimmed milk (12 g dry skimmed milk/ 100 ml of 0.01 mol/l calcium chloride) at 40 °C and the clotting time was recorded. The enzyme activity that clotted 10 ml milk in 10 min was taken as unit.

Estimation of protein. This was done by the method of LOWRY and co-workers [7].

Examination of the effect activators and inhibitors, urea and animal sera on milk-clotting activity. Enzyme dissolved in 2.5 ml (0.02 mol/l acetate buffer, pH 4.63), containing the test substance was incubated for 10 min at 40 °C. Thereafter, the enzyme solution was further incubated at 62 °C with 10 ml reconstituted skimmed milk and the clotting time recorded.

Animal sera. These were obtained from Serum Department, Virus Research Production Centre, Agouza, Cairo.

Amino acid analysis of the rennin-like enzyme. Enzyme sample was hydrolyzed in 6 N-HCl in a sealed tube for 24 h at 100 °C. The hydrolysate was chromatographed on Whatman No. 1 paper using the 2-dimensional technique with the solvents n-butanol — acetic acid — water (4 : 1 : 5, v/v) and phenol — water (80 : 20, mass/v). For tryptophan, the enzyme sample was hydrolyzed with 14% (mass/v) $Ba(OH)_2$ in an oil bath at 125 °C under reflux for 18 to 20 h. After removal of barium with 1 N-H_2SO_4 followed by washing the precipitate with hot water, the filtrate was concentrated in a vacuum oven at 35 °C. Paper chromatography of the hydrolysate was performed with a solvent consisting of isopropanol–ammonia–water (80 : 5 : 15, v/v). Spraying was carried out with ninhydrin.

Results and discussion

The fraction precipitated at 50% acetone from the partially purified milk-clotting enzyme preparation was analyzed by electrophoresis. It was shown that the fraction consisted of a single protein component which moved towards the cathode. This enzyme fraction represented the pure rennin-like enzyme (PRLE) preparation used in this work.

The milk-clotting activity of the PRLE was highly dependent on the enzyme concentration (Table 1). An enzyme concentration of 0.12 mg/ml was sufficient for maximal clotting. Using PRLE concentrations ranging from 0.02 to 0.14 mg enzyme protein in 1.0 ml, the clotting times were slightly shorter than the calculated values as expected from the preceding values containing lesser amounts of protein. Thus the clotting was accelerated with the increase of enzyme concentration. This indicates that no time-lag is required for the formation of the milk clot, as proposed by HOLTER [5], when suitable concentrations of the enzyme are used. This lag period seems thus to depend on the enzyme concentration and consequently on the rate with which the enzymic reaction product is formed.

Although proportional amounts of calcium chloride were added to milk, the rennin activity was inversely related to the substrate concentration supposedly due to the high viscosity at high substrate concentrations.

Table 1

*Effect of the enzyme protein concentration on the milk-clotting activity of PRLE**

Enzyme concentration (mg protein/ml)	0.02	0.06	0.10	0.12	0.14
Clotting-time (min)	31.08	7.78	4.13	3.22	2.82
Milk-clotting activity (unit/mg protein)	6.45	8.56	9.68	10.36	10.20

* PRLE = pure rennin-like enzyme prepared from *Penicillium citrinum*

The PRLE reached its maximal activity at 62 °C, but identical result was obtained at 67 °C. The rate of activity declined above the optimal temperature slowly, indicating resistance of the enzyme against heat in the presence of the substrate.

At any of the temperatures investigated, the enzyme showed maximal activity at pH 6.1 (Table 2). This value is slightly higher than those found for other fungal rennin enzymes [9, 13]. At higher pH values and different temperatures, the milk-clotting activity decreased gradually reaching limits which were always higher than those at pH 6.05. This indicated that the enzyme, in the presence of its substrate, was more stable at pH values above than below the optimum. The milk-clotting activity of the PRLE was high at pH 6.1 and 62 °C; the enzyme clotted 4 000 times its own weight of skimmed milk within 30 seconds. This indicates a high degree of purity; the partially purified enzyme could only clot 827 times its own weight of skimmed milk within 148 seconds at pH 6.0 and 60 °C [2].

The enzyme stability depended on the pH, the temperature and also, on the duration of the treatment (Table 3). At low pH values the enzyme was unstable. The milk-clotting activity was destroyed when heated at 40 °C for

Table 2

Effect of the pH at different temperatures on the milk-clotting activity of PRLE

pH of enzyme solution	pH of reaction mixture	Temperature of reaction (°C)			
		30	40	50	62
		Milk-clotting activity (unit/mg protein)			
3.42	6.00	1.11	2.22	4.13	18.87
4.05	6.05	4.03	8.89	17.86	52.63
4.63	6.10	6.60	11.63	23.53	66.67
4.99	6.20	5.10	10.31	21.98	57.14
5.37	6.20	5.00	9.52	20.41	46.51
5.89	6.25	4.83	9.90	19.61	41.67

Table 3

The pH stability at different temperatures of PRLE

pH of enzyme solution	Temperature of heating (°C)	Time of heating (min)	Milk-clotting activity (unit/mg protein)	Relative activity (per cent)
Control	40	10	42.857	100.0
3.42	40	10	5.345	12.46
		20	NC	0.0
	45	5	NC	0.0
4.04	40	10	34.783	81.16
		30	17.746	41.41
		60	0.395	0.92
	45	5	19.041	44.43
		10	0.724	1.69
	50	5	NC	0.0
4.99	40	10	38.167	89.05
		60	28.625	66.79
		120	22.342	52.13
	45	10	25.096	58.55
		30	0.833	1.09
		60	NC	0.0
	50	5	7.182	16.75
		10	NC	0.0
5.89	40	10	58.988	137.63
		60	48.193	112.45
		120	33.619	78.44
	45	10	40.723	95.02
		30	14.241	33.23
		60	NC	0.0
	50	5	17.209	40.15
		10	NC	0.0

After each treatment, the pH of the enzyme solution was readjusted to 4.63 and incubated with milk at 62 ° C; pH of control = 4.63: NC = no clotting

20 min or at 45 °C for 5 min. Generally, the enzyme was more stable at pH 5.89 and gradually lost its activity with decrease of pH value; it was more stable against heat at high than at low pH values. An activation effect was gained when the enzyme solution of pH 5.89 was heated at 40 °C but not at higher temperatures for 10 or 60 min. Although the enzyme showed maximal milk-clotting activity at pH 6.1 and 62 °C, it was unstable at pH 5.89 and

50 °C, supposedly due to an autolysis of the enzyme in absence of its substrate.

Complete inhibition of enzyme action could be brought about by iodoacetic acid, cystine or iodine (Table 4). Treatment of the enzyme with maleic acid or potassium ferricyanide led to partial inhibition of the enzyme. On the other hand, cysteine hydrochloride, reduced glutathione, potassium cyanide or ascorbic acid showed slight inhibitory effects. In any case, these results do not provide conclusive evidence that the active site of the PRLE involves reactive sulfhydryl groups. No reactive -SH groups were found to be in-

Table 4

Effect of some activators and inhibitors on the milk-clotting activity of PRLE

Substance added	Molarity of substance in enzyme solution	Milk-clotting activity (unit/mg protein)	Relative activity (per cent)
Control		12.57	100.0
Glutathione	0.001	11.67	92.84
(reduced)	0.005	11.03	87.91
	0.010	10.07	80.11
Potassium	0.001	10.26	81.01
cyanide	0.010	9.06	72.06
Cysteine	0.001	12.57	100.0
hydrochloride	0.010	11.03	87.91
Ascorbic	0.001	10.46	83.38
acid	0.010	8.96	71.27
Maleic	0.001	12.01	95.55
acid	0.010	8.86	70.49
	0.020	3.98	31.69
Iodoacetic	0.001	10.67	85.02
acid	0.010	6.75	53.75
	0.020	NC	0.0
Cystine	0.001	11.42	90.84
	0.010	NC	0.0
Potassium	0.001	10.13	80.87
ferricyanide	0.010	8.19	65.16
Iodine	0.001	NC	0.0

See Material and method for experimental conditions: pH of control = 4.63: NC = no clotting

Table 5

Effect of urea at pH 4.63 on the milk-clotting activity of PRLE

Molarity of urea in enzyme solution	Milk-clotting activity (unit/mg protein)	Relative activity (per cent)
Control	12.57	100.0
0.001	11.58	92.12
0.010	10.96	87.19
0.500	9.01	71.67
1.0	7.68	61.10
2.0	NC	0.0

pH of control = 4.63: NC = no clotting

volved in the active sites of other rennin enzymes [4, 10]. On the other hand, since the substrate of PRLE is also a protein (casein), this may also react with such agents.

Treatment of PRLE with 2 mol/l urea at pH 4.63 led to its complete inactivation (denaturation) (Table 5). In this respect, the PRLE is similar to animal rennin [4]. CHEESEMAN [4] attributed the loss of enzymatic properties by rennin in urea solution to the reaction of susceptible groups, i.e., tryptophan and tyrosine residues, to the environmental conditions and not to the extent of unfolding of the protein molecules. In the present work, the PRLE was found to be composed of 17 amino acids, viz., cysteine, aspartic acid, gluta-

Table 6

Effect of some animal sera on the milk-clotting activity of PRLE

Serum added	Serum protein/ 2.5 ml enzyme solution (mg)	Milk-clotting activity (unit/ mg protein)	Relative activity (per cent)
Control	—	33.67	100.0
Calf	4.20	28.06	83.33
	42.00	NC	0.0
Horse	4.40	24.69	73.32
	22.00	NC	0.0
Human	6.10	31.66	94.03
	61.00	NC	0.0
Rabbit	6.10	25.72	76.38
	12.20	3.14	9.32

pH of control = 4.63: NC = no clotting

mic acid, serine, glycine, threonine, alanine, arginine, lysine, histidine, tyrosine, methionine, valine, leucine, isoleucine, phenylalanine and tryptophan. The presence of tyrosine and tryptophan among the amino acids composing the PRLE may support CHEESEMAN's view [4].

Some animal sera were found to inhibit the milk-clotting activity of the PRLE (Table 6). Comparatively, human serum seemed to be less inhibitory than calf, horse or rabbit serum. These results are consistent with those found for animal rennin [8, 11, 12] and the milk-clotting enzyme of *Mucor pusillus* LINDT [6]. It is noteworthy that the inhibitory substances in such animal sera may affect the milk and/or the enzyme and hence the reaction. In the present work, the enzyme was incubated with the serum before addition to milk.

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Acta biol. Acad. Sci. hung., **24**(1—2), 103—118 (1973)

DEVELOPMENT OF IDIOBLASTS AND RAPHIDES IN THE AERIAL ROOT OF MONSTERA DELICIOSA LIEBM

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(Received; 1972—09—27)

Abstract

The idioblast initials are formed in the outer part of periblem close the tip in the aerial root of *Monstera deliciosa* LIEBM. The idioblasts can be 20 to 40 times longer than the surrounding cells. The developing and functioning idioblast contains a large nucleus and the cytoplasm is rich in organelles being characteristic of the secreting cells. The number and ratio of organelles change during the development. In the cytoplasm of young idioblasts the initials of needle-shaped crystals of Ca-oxalate and the joinings of the crystal initials are produced by special connections of vesicles and tubuli of dictyosomes and endoplasmic reticulum. In the developing idioblasts needle-shaped crystals may be formed also directly without appearance of crystal initials at the joinings of membranes. The growing crystals are ordered in rows between the membrane pairs. New crystals may adjoin these rows, and what is more, newer crystal centres may be formed in the cytoplasm. The cell-sap vacuole occurs somewhat differently from those being in the surrounding cells. In the idioblast it comes into being after the appearance of crystals, and the raphides getting in it may grow further. The cytoplasm of the idioblasts filled in with crystals is gradually degenerated upon the influence of lysosomes.

Introduction

The clarification of induction and development of crystals found in great number in different organs of lower and higher plants, among them, those of needle-shaped crystals of Ca-oxalate, represents even now an unsolved problem of cytology, physiology and cytoecology. It has not been cleared up till now how and why these crystals are formed. We have studied the cytomorphology of crystal formation.

The needle-shaped crystals are formed in idioblasts. FRANK [7] observed in light microscope that the crystal-idioblasts appeared after the equal division of cells. According to KOWALEWITZ [9], the idioblasts often contain two nuclei. SCHÖTZ and co-workers [19] observed by electron microscopy that karyokinesis was always followed by cytokinesis in the idioblasts.

On the basis of light microscopic investigations it is believed that the crystals may be formed within the cell, or in the case of Ca-oxalate of not raphide type, even in the intercellular space [see for details 22]. The intracellular appearance of crystals take place either in the cytoplasm [8, 18], or the crystals remain in the cytoplasm only during the early stage of their development

and later they get in the vacuole [14], or it is formed directly in the cell-sap vacuole [4, 17, 21], or occasionally they come into being in a small vacuole independent of the cell-sap [10]. However, the tonoplast of the latter type of vacuoles cannot be demonstrated by light microscopy [7].

Electron microscopy failed to solve the contradictions regarding the formation of crystals. While some authors are of the opinion that the raphides are brought about in the cell-sap vacuole [11, 13, 19] and even complete druse crystals have been observed in the cell-sap [16], according to WATTENDORF [22], the Ca-oxalate crystals are formed in the cytoplasm within a cellulose capsule and remain there.

The electron microscopy of crystal-idioblasts have revealed that the crystals are ordered in rows between membrane-pairs [2, 3]. MOLLENHAUER and LARSON [13] emphasized the secretory activity of the idioblasts, and attributed a greater significance to the carbohydrate matrix in the crystal formation than to the surrounding membranes. On the contrary, ARNOTT [2], PAUTARD [15], SCHÖTZ and co-workers [19] believe that the membrane within the vacoules are of importance in the crystal-formation. The crystal formation is attributed to the raphidosomes by LEDBETTER and PORTER [11], organized from the tonoplast and ordered in rows within the vacuoles, determining the crystal orientation.

Material and method

According to our observations both the initial idioblasts and the developing ones occur in the peripheral periblem of growing aerial roots of *Monstera deliciosa*. Therefore, the tip and the terminal part of about 2 cm of growing aerial roots of 10—30 cm length and 5—7 mm thickness were used for our investigations.

For light microscopy the material was fixed in Bouin solution, embedded in paraffin. Longitudinal sections of 10—12 μm thickness were cut and stained with hematoxylin dissolved in 70% ethanol.

For electron microscopy the tip of the aerial root and the periblem ot the terminal zone, at about 0.5 cm distance were used. According to the method of SCHÖTZ and co-workers [19] the material was fixed in 2% $KMnO_4$ buffered with veronal-acetate (pH 7.2) for 2 hours at room temperature, dehydrated in ethanol, embedded in Durcupan ACM and cut on a Porter-Blum ultramicrotome by glass knives. The sections were contrasted with uranyl acetate and lead citrate. Electron micrographs were taken in a KEM-I type electron microscope (GDR).

Results

The polyarchic aerial root of *Monstera deliciosa* is formed endogenously from the shoot. The idioblast initials are brought in the outer periblem both in the root tip and a little further in the vacuolated zones. However, in the latter, one can find even needle-shaped crystals containing idioblasts of different development and size.

The appearance of the idioblast initial is preceded by an unequal division. One of the two daughter cells, being larger than the other, shows a quick

vacuolation and becomes a typical periblem cell. The other, smaller, daughter cell being the initial of the idioblast is vacuolated later than its surrounding, it has a rich cytoplasm with numerous organelles and grows quickly. The young idioblast which contains already crystals is characterized by a large nucleus (Fig. 1). It may occur that the karyokinesis (mitosis) of the idioblast is not followed by cytokinesis, in such cases the cell contains two nuclei.

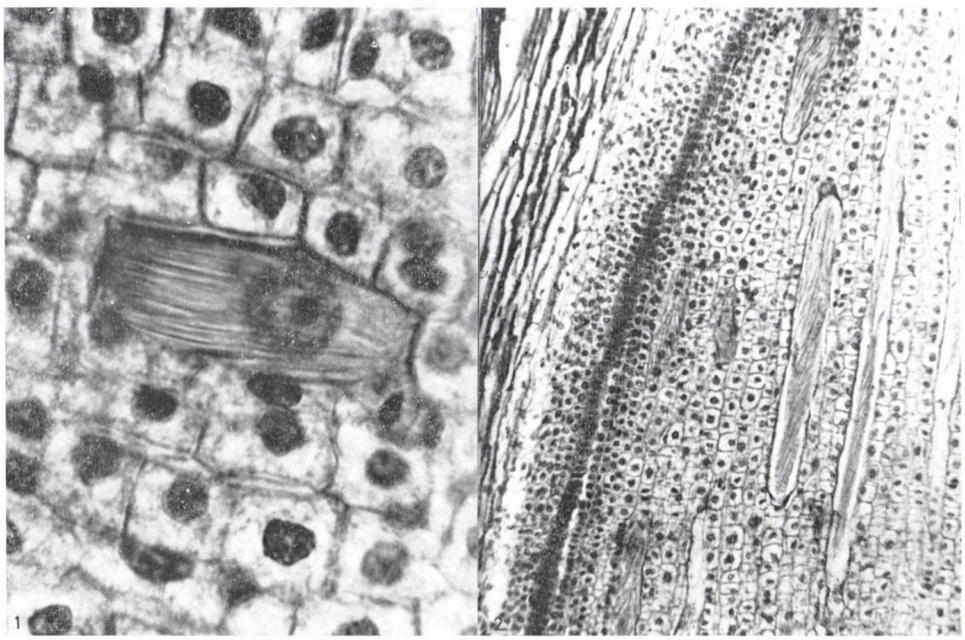


Fig. 1. Developing idioblast with large nucleus and needle-shaped crystals in the outer part of the periblem. Longitudinal section; ×500
Fig. 2. Idioblasts from the zone near the tip of the aerial root. Detail of a longitudinal section; ×75

The idioblasts grow mainly parallel with the regularly oriented longitudinal rows of the primary cortex, however, along the tangential walls, they become even wider at the beginning. They grow 20 to 40 times longer, but only 2 to 3 times wider, than the typical cortical parenchymatous cells (Fig. 2).

In the aerial root of *Monstera deliciosa* the boundles of needle-shaped crystals, i. e. the raphides consist of Ca-oxalate-monohydrate [1] (Figs 3a and b). According to STEINFINK and co-workers [20], this type of crystals do not come into being *in vitro*. The needle-shaped crystals cannot be determined optically [5, 6]. According to our measurements the crystals are 40 to 60 μm long and 1.5 to 2.0 μm wide, tapering toward the poles. The cross-section of crystal is roughly quadrangular. In the central zone of the growing crystals, ribs are to be seen on the longitudinal edges (Fig. 3b).

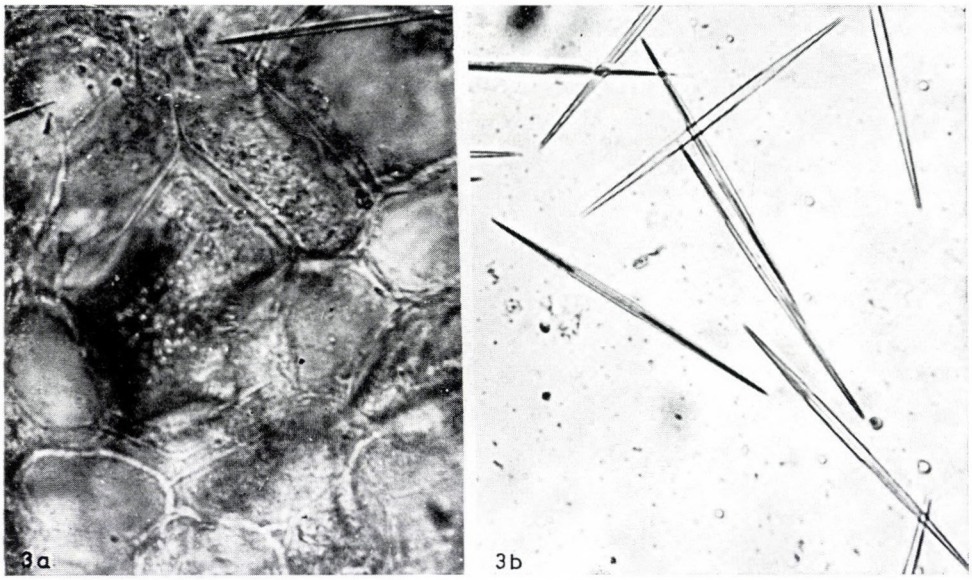


Fig. 3a. Cross-section of an idioblast with needle-shaped crystals; ×700
Fig. 3b. Needle-shaped crystals of Ca-oxalate; ×1 000

After the unequal division, as soon as the idioblast initial reaches the size of the surrounding cells of the periblem, formations of different section planes showing a definite outline as well as striation of electron density and opacity appear in several regions of the cytoplasm, which represent the crystal initials (Figs 4, 5a, 6a, 6b, 7a, 7b). The bright dark striae may be variable in direction width and contrast. Two to five rows of the crystal initials localized side by side in the same or different planes represent the raphide initials.

The young idioblasts are characterized by an extremely intense vesiculation (Figs 5a, 5b, 6a). The vesicles seem to be partly of pinocytotic ones being close the plasma membrane, partly of the products of dictyosomes and of the endoplasmic reticulum which surround semicircularly the dictyosomes (Fig. 5b). The membranes of vesicles are either paired (Fig. 5b) or connected like a collar of pearls (Fig. 5a), or may form tubuli by fusion (Figs 8a, 8b). The vesicles and tubuli may increase the size of crystals initials and raphide initials by gradual incorporation or attachment (Fig. 7a), as well as they may participate in the formation of new centres (Fig. 11). The crystal initials are composed either by the membranes of vesicles or by the substances transported, or by both of these.

The orientation of the raphide initials in the cytoplasm is roughly identical with that of the mature raphides. The Ca-oxalate has probably no crystalline form in the crystal initials, as it has been pointed out by ARNOTT [2] and

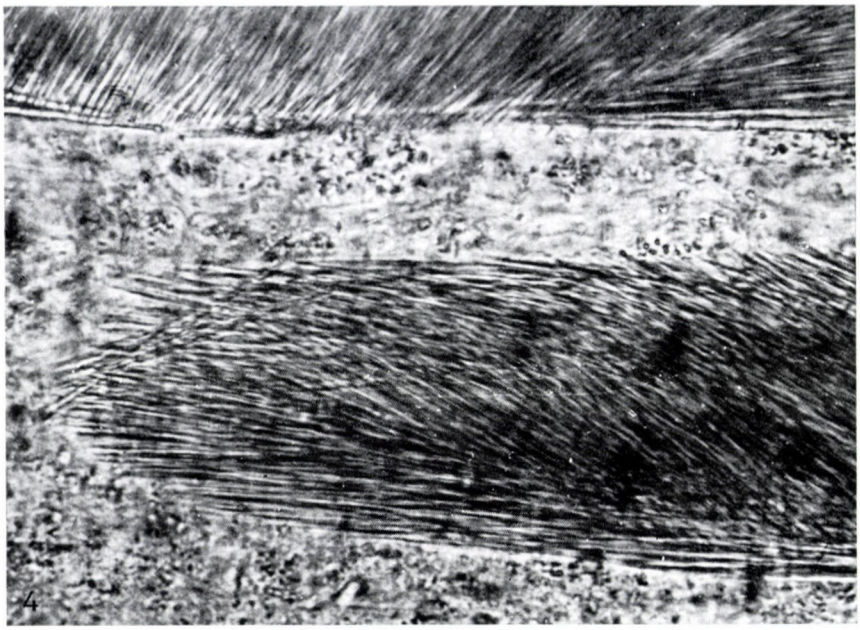


Fig. 4. Idioblast filled in with raphides. Longitudinal section; ×700

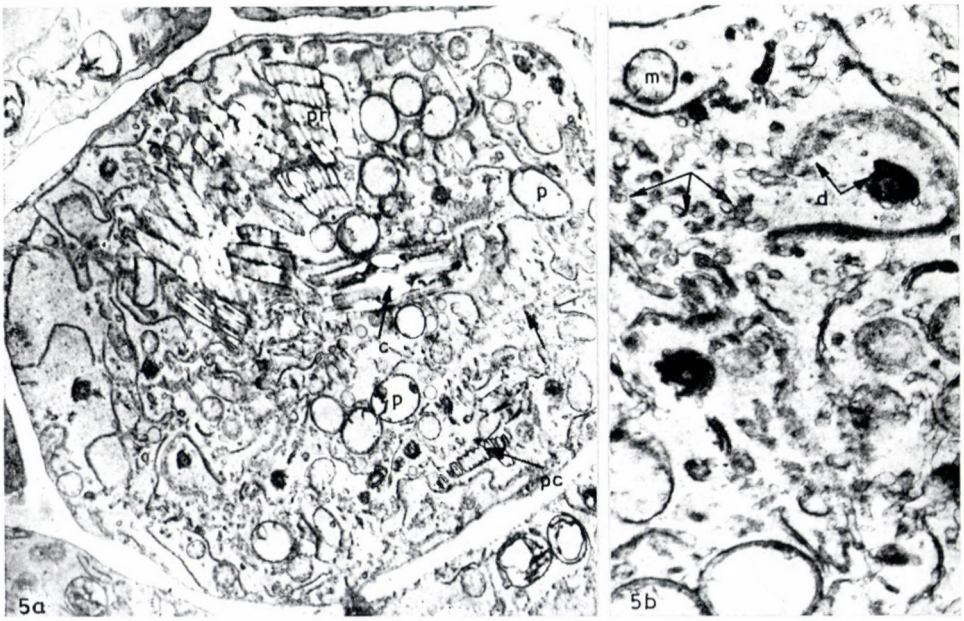
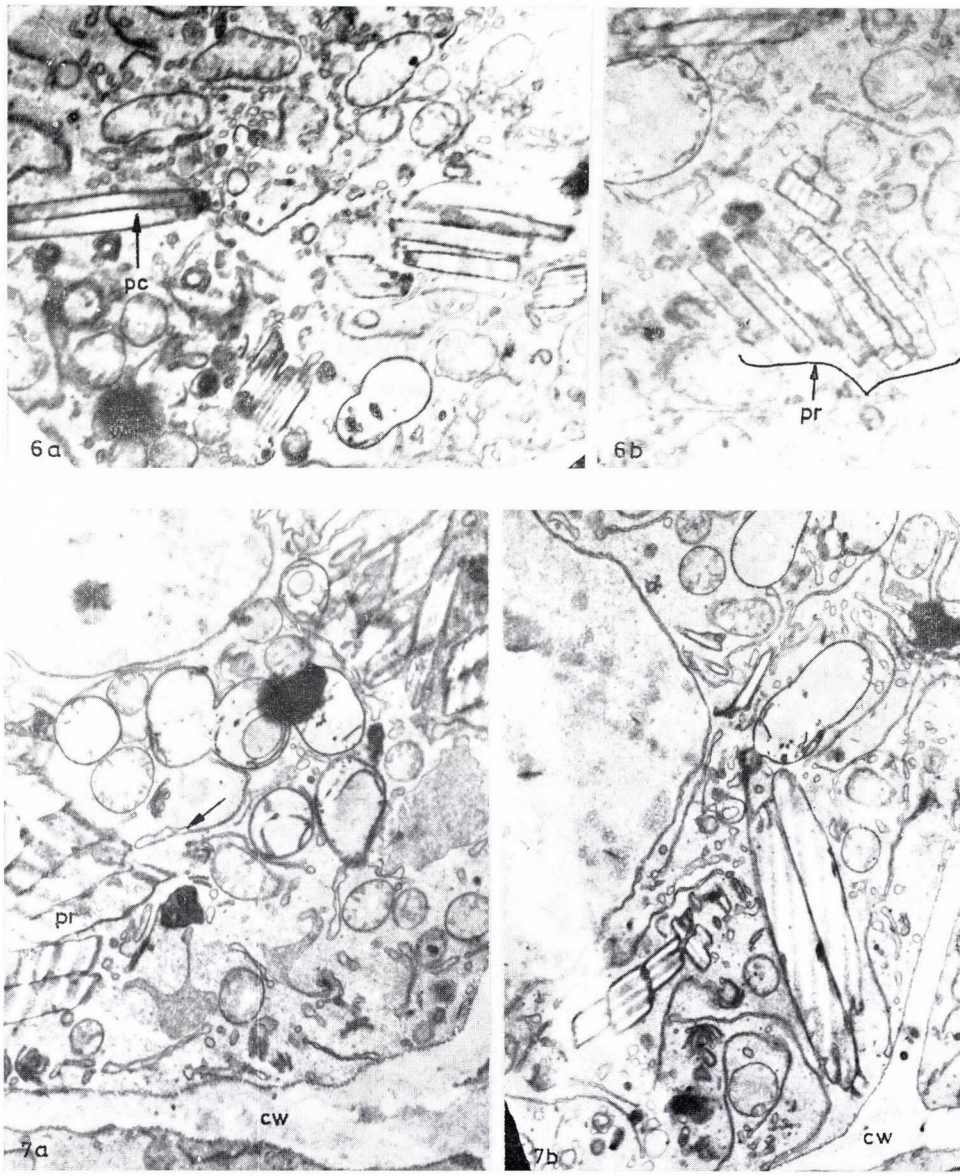


Fig. 5a. Idioblast initial with raphide initials; ×4 400
Fig. 5b. Detail of the cell shown by Fig. 5a; connections of vesicles, and endoplasmic reticulum surrounding semicircularly the dictyosomes: ×13 200



Figs 6a—b, 7a—b. Details of idioblast initials containing growing raphide initials; 6a = ×8 800, 6b = ×13 200, 7a and 7b = ×8 800

PAUTARD [15], and as we assume on the basis of striation of electron opacity observed in the raphide-initials; crystalline Ca-oxalate would be unpenetrable for the electron beam. As soon as the crystallization starts, the dark formations appear (Figs 9, 14a, 14b, 14c). When the crystals further increase, they usually

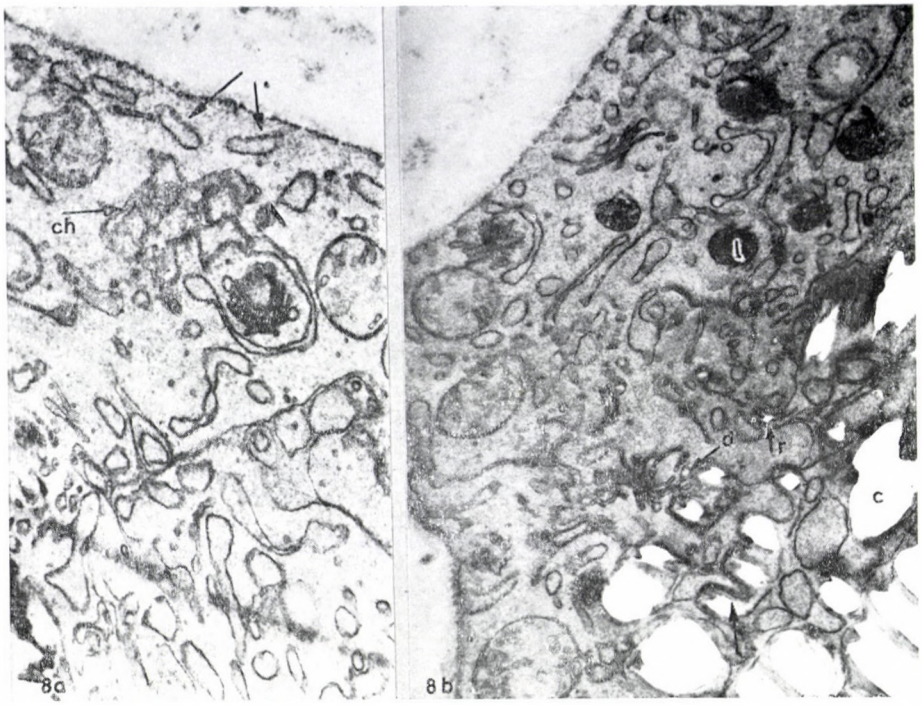


Fig. 8a. Detail of an idioblast with forming crystal-chambers; ×20 000
Fig. 8b. Detail of an idioblast: rows of crystals, lysosomes, needle-shaped crystal formation within dictyosomal vesicles. (The crystals cut perpendicularly had fallen out, their places are indicated by white holes); ×20 000

fall out during cutting and only white holes can be seen instead of them on the micrographs (Figs 8b, 9, 10, 13b).

The young idioblasts contain voluminous endoplasmic reticulum, many dictyosomes, mitochondria and proplasts with several lamellae (Figs 5a, 6a). The young and growing idioblasts have hardly any plasmodesmal connection with the adjacent cells (Figs 7a, 7b, 12, 13b, 14a, 14b, 14c).

During quick growing and intense secretion, the Ca-oxalate appears also in crystalline form. The crystallization begins in the raphide initials (Fig. 5a). The crystals organized later may be formed even in different ways, e. g. in the vesicles of dictyosomes (Fig. 8b), between the contacting, often undulated membrane surfaces (Figs 9, 10a), or at the boundary of larger vesicles (Fig. 9). The crystals are mainly ordered in rows between the membrane pairs within the cytoplasm (Figs 10b, 11). Even the single crystals are enveloped partly or totally by membrane.

The crystals do not reach their mature shape at once but they gradually grow. The growing may take place on the way described at the raphide in-

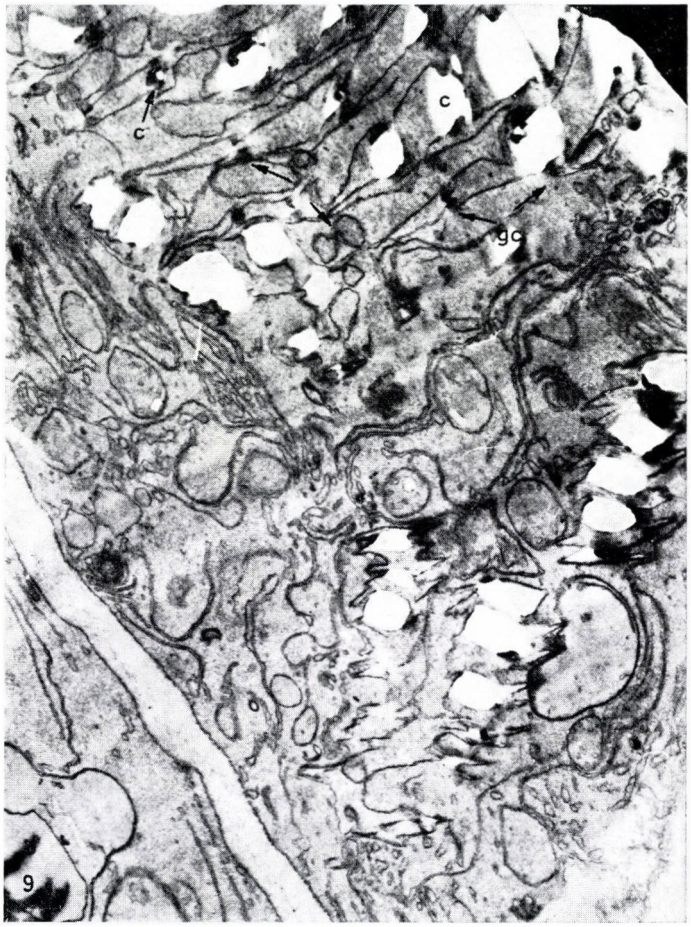


Fig. 9. Details of idioblasts with crystal rows forming in different way; × 9000

itials, or by penetration of the large vesicles and dense vesicle-chains found between the crystal rows, which presumably contain the components of the crystals, through the membranes delimiting the crystal rows (Figs 8b, 10b). New crystals may be attached to the crystal rows (Figs 9, 10b, 11), or even new crystal centres may be formed (Fig. 11). The dense Z and H formations of Fig. 9 and Fig. 14 represent the cross-sections of growing and developed crystals, respectively.

Apart from the crystal formation of various forms, even new raphide initials can be formed in the growing idioblasts. This is indicated by the association of numerous wide tubuli with undulated membranes in the cytoplasm (Fig. 11).

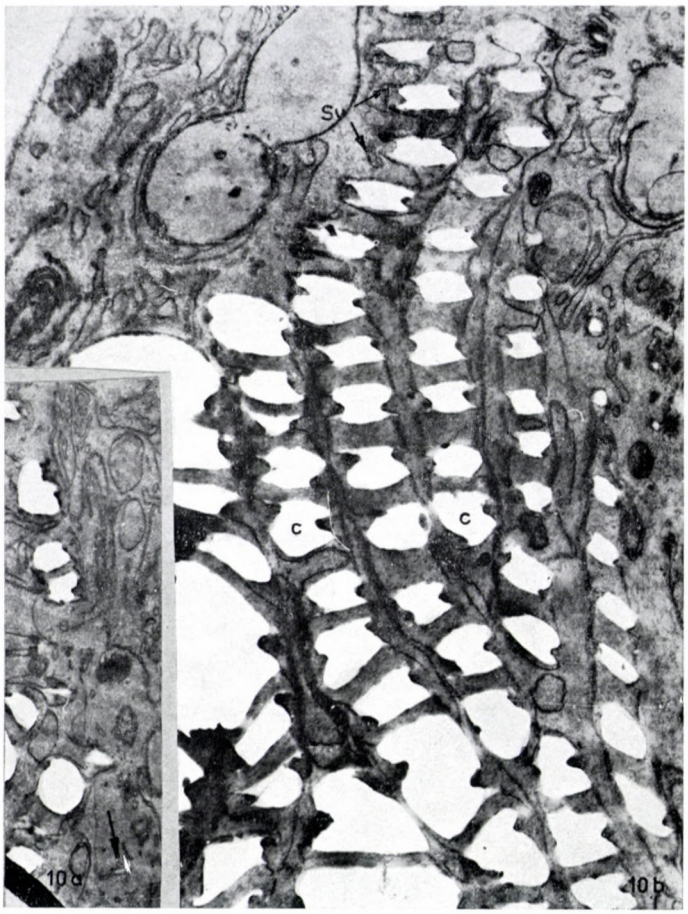


Fig. 10a—b. Details of idioblast with crystal rows; 10a = × 8800, 10b = × 17000

Parallel with the crystal formation, the dictyosomes and lysosomes greatly increase in number (Fig. 8b and the diagram). The dictyosomes appear in pairs, their cisternae are twisted in circles (Fig. 11). The increase in number of lysosomes indicates that their role in the life of cells also increases.

The young cells contain no vacuoles, whereas in the idioblasts of about 3-fold size the primary vacuoles appear because of widening out of the endoplasmic reticulum (Fig. 13b). Another type of vacuolation comes into being as a consequence of cytolysis. This is induced by the disintegration of lysosomes in the vicinity of crystals, taking place without an immediate formation to tonoplasts (Fig. 12 shows the cytolysis between the nucleus and cell wall in the cytoplasm devoid of tonoplast). The large cell-sap vacuole of idioblast is

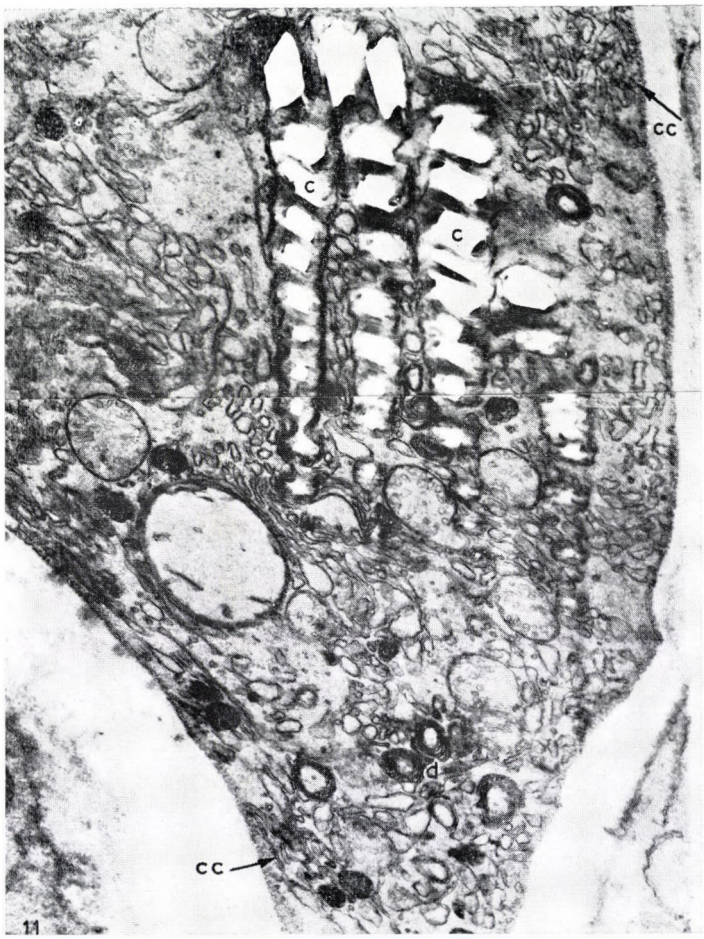


Fig. 11. Organization of rows and centres of crystals in the cytoplasm. Cell organelles are between the rows of crystals; ×13 200

formed by fusion of small vacuoles of endoplasmic reticulum origin and by the lysis of degraded cytoplasmic regions.

Together with other cytoplasmic ruins, also the needle-shaped crystals get in the large cell-sap vacuoles (Figs 13a, 13b). The membrane rows orienting the crystals may persist even within the vacuole (Fig. 14b), or they can be gradually dissolved (Fig. 14c). The vacuolation observed by us agrees with the observation of MATILE and MOOR [12] with the only exception that we observed cytolysis without formation of tonoplasts.

Together with the rapid growth of the idioblasts, accompanied by the increase of cytolysis, the cell-sap vacuole occupies a larger and larger space within the cells. The crystals can further grow within the vacuole by means

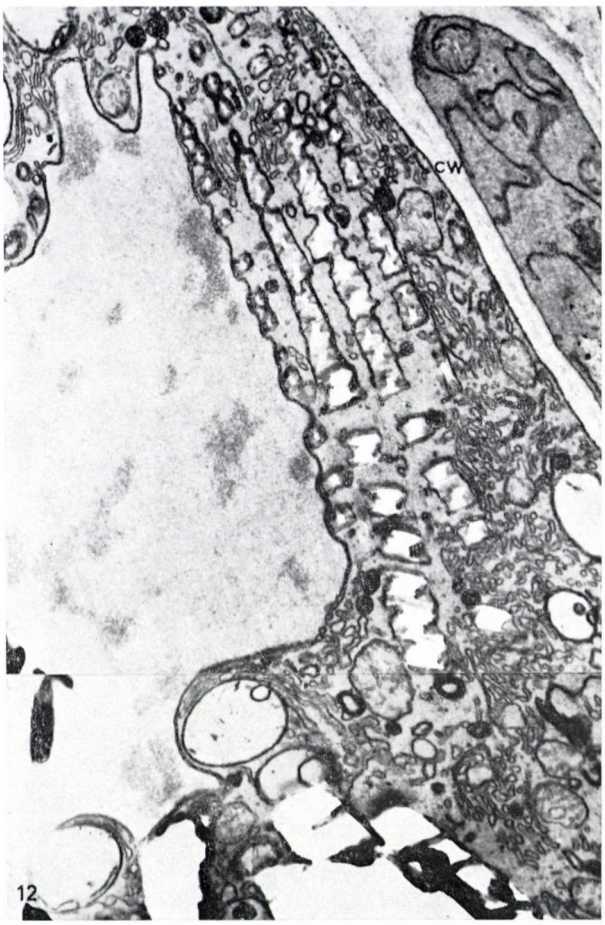
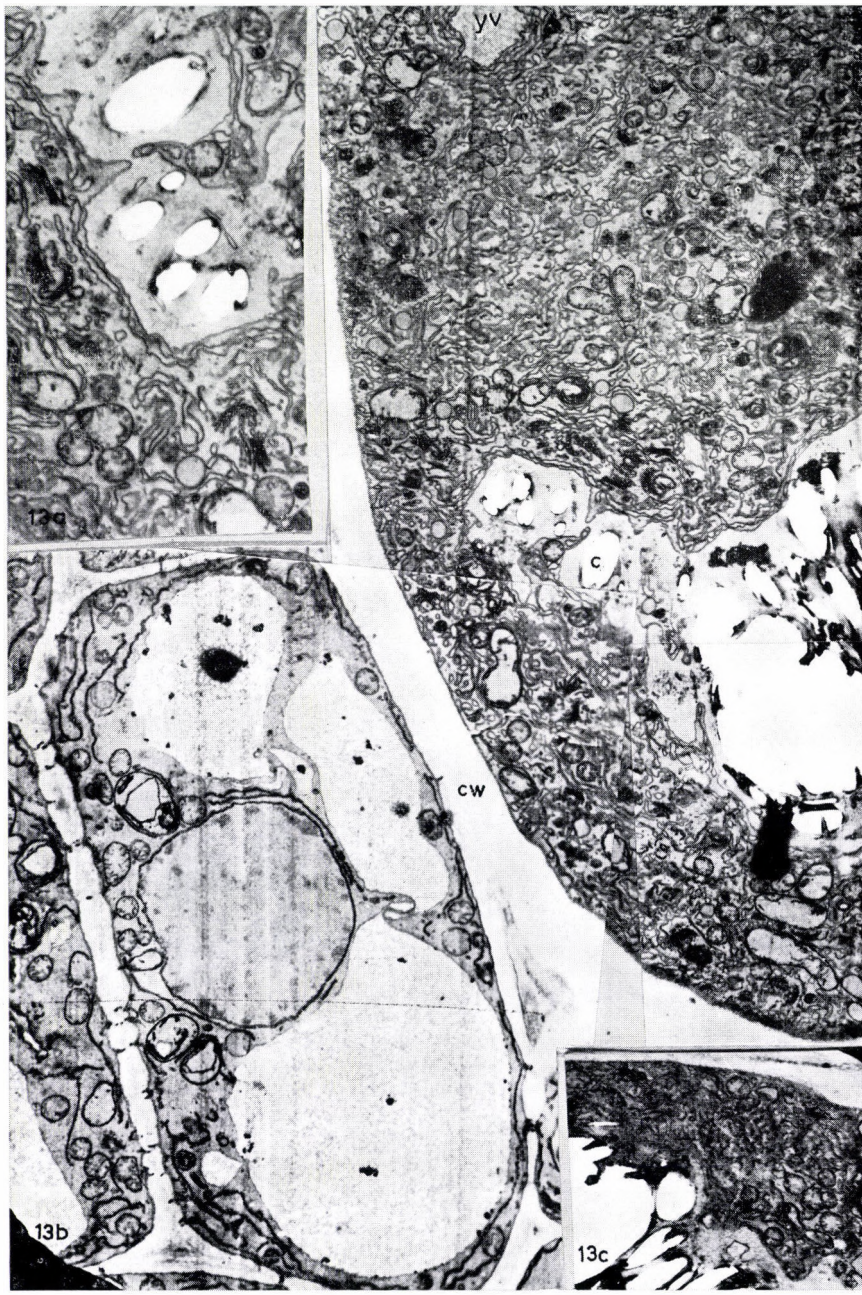


Fig. 12. Cytolysis and development of cell-sap vacuole close the crystal rows; ×8 800

of the substance transported by the endoplasmic reticulum. The tonoplast is disrupted at some places during the transport of substances and both the endoplasmic reticulum and its content get in the vacuole (Figs 13a, 14a).

Parallel with the growing of the cell and the secretion process, the lysis is continued and, by means of its progression, the endoplasmic reticulum is transformed into a vesicular one (Fig. 14b), i.e. it degenerates. In the last stage of cytolysis the cell organelles disappear, at latest the mitochondria and the vesicular endoplasmic reticulum, and the lysosomes digest the cytoplasm almost completely (Fig. 14c).

Considering the role of the cell organelles in the formation of Ca-oxalate crystals, we counted the dictyosomes, mitochondria and lysosomes in the growing idioblasts. It was found that there is a significant, positive correla-



Figs 13a—c. Details of idioblasts resembling secretory cells and an adjacent periblemal cell: crystal needles within the cell-sap vacuoles; 13a = ×8 800; 13b and 13c = ×4 400

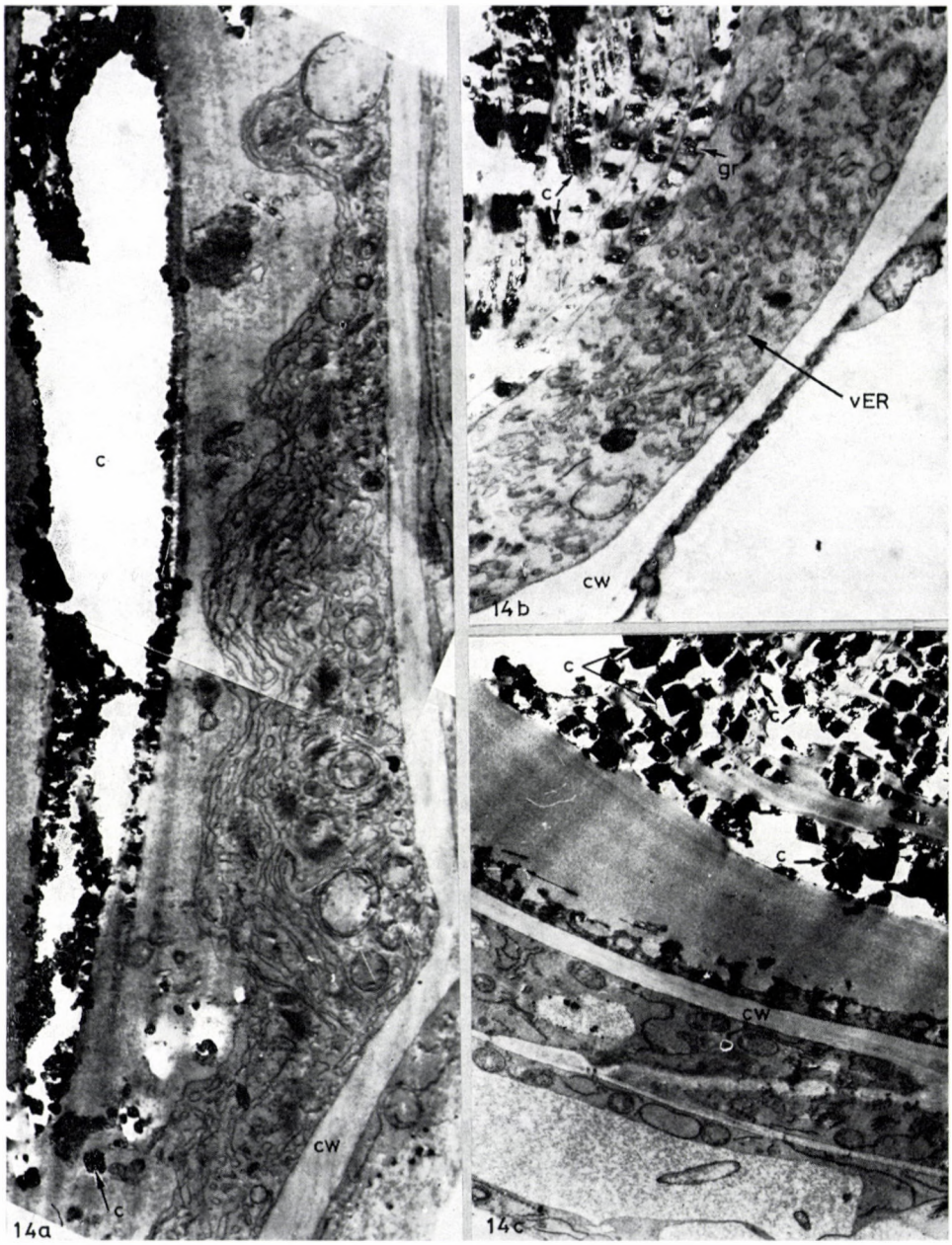


Fig. 14a. Detail of an idioblast with intensive secreting cytoplasm: longitudinal section of a needle-crystal in the cell-sap vacuole; ×8 800

Fig. 14b. Vesicular endoplasmic reticulum in the gradually degenerating cytoplasm and membrane-bounded crystal-rows in the vacuole; ×8 800

Fig. 14c. Detail of an idioblast, degenerating cytoplasm, vacuole filled in with crystals; ×4 400

c = crystal, ch = crystal chambers, cw = cell wall, d = dictyosome, ER = endoplasmic reticulum, gc = growing crystals, m = mitochondrion, p = proplast, pc = primary crystal (crystal initial), ri = raphide initial, r = raphidosome, t = tubuli, v = vesicle, vER = vesicular endoplasmic reticulum, yv = young vacuole

tion between the number of dictyosomes and mitochondria, being better than 10%, however worse than 5. The positive correlation between the dictyosomes and lysosomes is significant at the 10% level. The mitochondria and lysosomes are not significantly correlated with each other (NS).

As it has been mentioned above, our investigations concerned only the idioblasts near the root tip. However, the idioblasts grow and secrete even further from the root tip until the cytoplasm remains able to function.

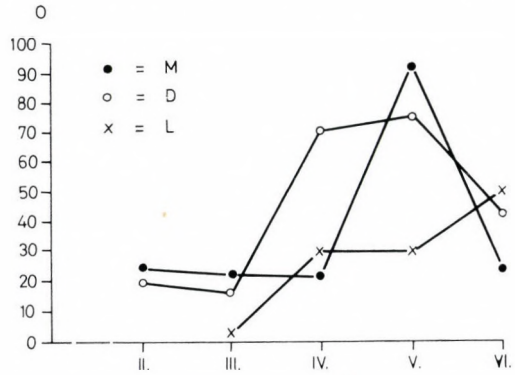


Fig. 15. Changes of the number of cell organelles parallel with the growth of idioblasts Abscissa: The increase of idioblasts in size related to the initial one. Ordinate: Changes in the number of cell organelles. M = mitochondrion, D = dictyosome, L = lysosome

Discussion

Unlike the other types of Ca-oxalate crystals, needle-shaped ones cannot be produced *in vitro* [20]. It can be assumed that their synthesis and secretion is enzymically regulated. Our work analyzed the cytomorphologic aspects of this type of crystal formation to be regarded as a secretion process. In agreement with WATTENDORF [21] and in contradiction to ARNOTT [2], MOLLENHAUER and LARSON [13], SCHÖTZ and co-workers [19], as well as LEDBETTER and PORTER [11], it has been found that the process of crystallization begins in the cytoplasm (Figs 5—12). Parallel with the development of the idioblasts and the formation of the cell-sap vacuole being connected with the cytolysis, the raphides and the whole process of crystal formation get into the cell-sap vacuoles. Comparing our material with the pictures of ARNOTT [2] taken from the root of *Yucca* and the axis of shoot of *Eichhornia*, with those of MOLLENHAUER and LARSON [13] taken from the aerial roots of *Vanilla* and *Monstera*, as well as with those of LEDBETTER and PORTER [11] taken from the mesophyll of *Spirodela*, one can establish that those authors investigated only a very late phase of the crystallization (see Figs 14a, 14b) and this was the reason why they could find crystals only in the cell-sap vacuoles. FREY-WYSSLING

[6] assumed that the Ca-oxalate could occur as a liquid phase, too, or in the form of crystals hydrated in different degree, and it is transformed in the solid stable monohydrate state by means of gradual dehydration.

According to our observations, the Ca-oxalate crystals are ordered in rows between the pairs of membranes in the growing idioblasts, just as described by ARNOTT and PAUTARD [3] as well as by LEDBETTER and PORTER [11]. Nevertheless, those authors observed crystal rows only within the cell-sap vacuoles, while we found them even in the cytoplasm (Figs 9, 10b, 11, 12). According our investigations the crystals get into the cell-sap vacuoles during the growing of the idioblasts and their membranes persist for a short period of time (Fig. 14b). A significant role has been attributed to the membranes in the crystallization by ARNOTT [2] as well as SCHÖTZ and co-workers [19]. They are of the opinion that the crystals are formed only in small chambers formed within the vacuoles. We also regard the membranes as important elements of secretion. However, this process is not bound exclusively to the chambers and takes place not only within the cell-sap vacuoles, but each membrane in the cytoplasm, even those of the organelles, participate in the formation of crystals. We found also the small chambers described by SCHÖTZ and co-workers [19] within the vacuoles, even in the cytoplasm (Fig. 8a). According to LEDBETTER and PORTER [11], the crystallization is performed by raphidosomes, also within the vacuoles. Such raphidosomes were also found by us in the cytoplasm (Figs 12, 8b), and we regard them as cross-section of growing crystals.

Our paper presents only the cytomorphological description of crystallization. However, it needs further elucidation how the calcium and oxalic acid are transported to these cells and how they are formed. MOLLENHAUER and LARSON [13] assume a slow, gradual crystallization within the vacuole, resembling the theory of FREY-WYSSLING [6]. According to LEDBETTER and PORTER [11], the oxalic acid freely penetrates the membranes, even the plasma membrane, and the raphidosomal membranes participate in the crystallization like a Ca-pump. The importance of this theory would be increased by proving that the Ca participating in the crystallization may originate not only from the raphidosomal membranes but from any other membrane of the cell. Nevertheless, it is possible that the oxalic acid is connected to other compounds or gets in the idioblast as another compound, and the Ca-oxalate formation is preceded by a long sequence of reactions. The intense cytolysis accompanying the secretion in the idioblasts can perhaps be explained by an accumulation of oxalic acid within the cytoplasm.

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RECENSIONES

VOGEL-ANGERMANN; **Taschenbuch der Biologie. Vol. 1—2.**
VEB G. Fischer, Jena (1972), pp. 570, DM 13.

This book was originally published in the FRG in 1967 and now revised in some aspects, republicated in the GDR. The parts of Vol. 1 are: cytology-histology-organology: the main types of living beings; multiplication; ontogenesis; ecology and taxonomy. Vol. 2 involves: metabolism; hormonal regulation and neural functions; movements; ethology; inheritance: evolution including that of man. In both volumes only the pages of odd numbers are for the text, while those of even numbers show the figures. The text is surprisingly compact involving a rich material of data in spite of its small volume. The work is characterized by very sharp definitions and strong proportions emphasized even typographically. The text follows each basic phenomenon of life, where it is possible at all, from the bacteria to man. This book resembles an encyclopaedia in construction, however, not in alphabetical order but according to the internal logic of natural science. Nevertheless, the subject index of the book outweighing 10 per cent of the book allows us to use it even as an encyclopaedia. The documentation of the book is well-selected, it involves coloured, sometimes too coloured, very didactic, schematic drawings which are mainly not of illustrative character but always of instructive significance. The compact text is not easy to read, therefore, it can be recommended first of all not for the entirely beginners. It will offer great help for the middle-school teachers and specialists who want to get quick and reliable information about the most essential concepts and data of certain interdisciplinary fields. At last but not least, both the selection of the material and the style emphasize the features of common interest without reducing scientific exactness, thus stimulating both the beginners and specialists for further reading. The only disadvantage of the book is perhaps that it has no list of references and even in the text there are only scattered references to literature.

T. ÁCS (Budapest)

WUNDERLI, J.; **Die Biologie des Menschen**
S. Karger, Basel (1973), pp. 192, Figs 30, SFr 24.

The book is an "Einführung "indeed, in the proper sense of the word. It is exclusively concerned with human biology and endeavours to disclose structure and physiology of the human body. Hence, perhaps its title should have been "Anatomy and physiology" like BÜCKER's book, which first appeared in Hamburg in 1939 and since that time has been constantly re-edited.

The book serves as a text-book for the teaching of nurses, but proves to be most useful also for the students of secondary schools and even for self-educated, as verified by the second edition, which appeared as soon as three years after the first one.

The division of the book is similar to that of other textbooks dealing with this matter: cells, connective and adipose tissue, locomotor organs, alimentation and digestive system, kidney, blood, heart and blood circulation, respiration, nervous system, sense-organs, hormones and sexual function. The context is concise, but of scientific character and up-to-date.

The main value of the book is the abstraction manifesting itself in the diagrams and the line-drawings easily reproducible by the student. This proves that the simplified rendering, aiming at essentials, the occasionally almost vulgarized, but by all means didactic representation customary in English text-books has — at last — been adopted in the German textbook literature. The coloured illustrations, though being of the usual comprehensive nature to

be found in every textbook (skeleton, musculature, etc.), with their presentation still reflect the up-to-date way of seeing and representing things. Some of them are even of artistic value (teeth, eye, ear).

Dr. WUNDERLI, with this work has succeeded in conveying such elementary biological notions to those concerned with this matter, relying upon which the future nurses, when having finished their further clinical and clinico-methodological training, might become most efficacious collaborators of the physicians in hospital wards.

The perfect typographic presentation of the book and its easy handling, the excellent colour-plates are a credit to the publisher: Karger.

T. DONÁTH (Budapest)

HOLLINGSWORTH, M. J., BOWLER, K.; **Principles and processes of biology**
Chapman and Hall Ltd. London (1972), pp. 457, £ 5.0.

This is a book of special interest in Hungary. It was more than two decades ago that general biology had been included into the curriculum of medical students in this country. It was presumed that students need a general integrated survey on biological sciences before they enter their more detailed studies of specialities. General Biology was intended to cover the common natural laws of the living world obtained in zoology, botany, microbiology, anthropology, evolution, systematics and ecology.

This task to fulfil was approached by the publishing of General Biology Textbooks for medical students in 1956, 1966 and 1970. It is interesting to compare the Biology (Publisher Medicina, Budapest, 1970) with HOLLINGSWORTH's and BOWLER's book of 1972 to see that in both cases the authors in principle came independently to a very similar conclusion when building up the structure of their textbooks.

The book under review consists of twelve chapters. It starts with an introduction to the chemical composition of living organisms, then proceeds to the cell level to demonstrate the natural laws in the "unit of life". The next chapters are about the general and characteristic fundamental functions of living state; the synthesis of proteins; the mode of energy production by living organisms; nutrition, this chapter includes photo- and chemosynthesis, heterotrophism and the secretion and excretion processes; growth and division of individual cells, population growth, multicellular organization, the control of growth, the aging and death; the reproduction and development with the summary of current knowledge about the mechanism of gene action and nuclear-cytoplasm interactions; irritability and integration that covers sensitivity, reaction, communication and co-ordination in both plants and animals; the living organism and its environment has a comparatively ample space in this condensed book, which is understandable considering the importance of the question; the last chapter is concerned with genetics, heredity and variation, forces and processes that are working in the evolution.

The authors were able to condense the vast material into four and a half hundred pages, a merit that is difficult to overestimate. The university students of many biological fields and also specialists in their postgraduate life who had started their studies one or two decades ago may get a clear and up-to-date picture of the fundamentals of their basic science: Biology.

G. SZABÓ (Debrecen)

Readings in aquatic ecology
Ed. FORD, R. F. and HAZEN, W. E. W. B. Saunders Co., Philadelphia—London—Toronto (1972), pp. 397, £ 2.90

This book is a collection of 29 separate papers each representing an important field of research in marine and fresh-water biology. The volume as a whole gives a good idea of the problems and techniques in modern aquatic ecology, the papers are divided into six parts.

Part I. Physiological and behavioral ecology. The photosynthesis of a marine diatom under laboratory and field conditions and nitrate uptake of marine phytoplankton were studied. Two papers deal with feeding, respiration and assimilation of marine and fresh-water crustaceans.

Part II. Small-scale distribution and sampling problems. This part is recommended to zoologists interested in the sampling error due to patchiness and avoidance of towed nets by zooplankton.

Part III. Population ecology. It comprises papers on the dynamics of natural populations of snails and crustaceans. The effect of predation on the body size and composition of plankton was demonstrated.

Part IV. Community and ecosystem ecology. Factors controlling phytoplankton populations were investigated. Primary production in coastal sea water was measured using large volume plastic sphere. In a reservoir chlorophyll content per unit volume of cells and photosynthesis per unit chlorophyll decreased with increase in population size. Community metabolism in terms of energy flow was studied in a temperate cold spring.

Part V. Biogeochemical cycles. It deals mainly with the nitrogen and phosphorus cycle of the sea.

Part VI. Aquatic pollution problems. Of the many types of pollution only the accumu-ation of plant nutrients and thermal pollution are treated in this short section.

S. Herodek (Tihany)

Schmidt-Nielsen, K.; **How animals work?**
Cambridge University Press, London (1972), pp. 114, £ 0.80.

There can be many approaches to the comparative physiology, however, if somebody is interested in the principles, the most excellent idea is to analyze the interrelationships of body size and form representing primarily perceptible realities to the effective ability. This implies the knowledge of energy consumption and the possibilities or limitations of the environment, thus we can better count or predict the work produced by animals.

K. Schmidt-Nielsen studies the working of animals in a wider interpretation from a physiological point of view. Breathing, flying, swimming and running are different kinds of working. The author describes the deeper bioenergetic background of these processes and elucidates the function of the effector organs, using analogies of machineries. Unfortunately, the function and structure of ribs of radiator, evaporating trays or climatic equipments are perhaps easier to understand for the modern people than e.g. the marvellous respiratory system of birds. The author subtly pictures that the machineries are only imitations and more primitive as compared to the biological systems, since they are devoid of abilitiy of adaptation. The reader must not reproach the author for the rather numerous mechanical analogies, but it should be attributed to our insufficient biological knowledge and our "homo technicus" way of looking at things calling for the help of technical elucidations.

The most valuable chapter of the book, according to the opinion of the reviewer, is that concerning the body size and unit of measure. This summarizes the problem mentioned above, namely that neither the living beings represent exceptions as regards the "ultima ratio" of mass and energy connections, on the contrary, they are involved in the great law during their life.

Gy. Fábián (Gödöllő)

Binyon, J.; **Physiology of Echinoderms**
International Series of Monographs in Pure and Applied Biology Division: Zoology Vol. 49.
Pergamon Press, Oxford—N. Y.—Toronto (1972), pp. 264, £ 4.80

Binyon's book is a comprehensive review of facts and ideas on physiology of Echinoderms. Most of the members of this group exhibit radial symmetry which is not a very common feature amongst Invertebrata. Profoundly affected in their organization by pentamerous condition they are characterized by such anatomical peculiarities as the lack of head and of true circulatory system, etc. It is not surprising, therefore, that their functions are highly modified.

The subjects of the volume are arranged in eleven chapters, providing detailed information on the physiology of the phylum. An abundant list of references, a special Addendum containing the most recent publications and an exhaustive index complete the book.

The introductory chapter deals with the problems of feeding and digestion. It is followed by parts providing information ont he excretion and the role of amoebocytes, on the salinity tolerance, osmoregulation, ionic regulation and chemical composition. Special chapters

are devoted to survey data on comparative biochemistry, toxins and immunology of the phylum. In the second half of the book the problems of sensory physiolcgy, respiration and locomotion are considered. Detailed information is given on spawning and neurosecretion, water vascular system and on physiology of nerves and muscles. In all chapters the discussion of physiological data is preceded by a brief description of the morphology of the given system, which greatly facilitates the reading for those not familiar with echinoderm anatomy. The volume is well illustrated, its technical presentation is excellent. The work is most useful for physiologists and for all those interested in comparative anatomy and zoology.

J. Kovács (Budapest)

Reproduction in mammals. Reproductive patterns. Artificial control of reproduction
Ed. Austin, C. R. and Short, R. V. University Press, Cambridge (1972), pp. 156 and 152, £ 1.30 and 1.30

In spite of that the titles suggest something else, even the last two volumes of this series deserve interest from both biologists and physicians, similarly as the former three volumes.

In the fourth volume the chapter concerning the species differences of the reproduction can, of course, point only to several characteristic examples, since the limited extent does not allow more. However, the examples are well selected. Thus it concerns the reproduction of marsupials and elephants as well as the problems of horses and their hybrids. The sexual behaviour stands now in front of the biological research: the second chapter can offer something from this field just as a sample, nevertheless this is done very practically and in a modern way. The third chapter gives a wide orientation about the environmental factors, emphasizing mainly the nutritive and social factors in cases of animal populations. The immunological chapter concerns first of all the biological factors playing a role in the masculine and feminine sterility, underlining particularly the causality and the problem of permeability of immunoglobulins from the mother to the foetus. The chapter of ageing attempts to give a survey of this important question, using excellent documentation and describing animal experiments.

The fifth volume contains 6 papers. The first of them deals with the technique of artificial insemination and questions of embryo transplantation. The second one concerns the problems of human reproduction potential, involving the questions of the ages of pairing and the intergestation periods, and evaluating critically the methods of reproduction. A separate chapter covers the masculine contraception and describes the present difficulties of this problem. A further paper describes the methods of obtaining human ovules and their *in vitro* cultures which is the primary work of the author (Edwards). It concerns the possibilities of recognition of the foetal genetic injuries and the problem of selective abortion. A separate chapter deals with the demographic problems of human reproduction and treats in detail the relationships of the Church and natural sciences from this point of view. The book is closed by the short but noteworthy chapter of Austin regarding the ethical problems of interference into the reproduction.

Similarly as previously, the presentation of the volumes is excellent. The whole series can be regarded as an attractive textbook offering which is just needed at a high level.

G. Csaba (Budapest)

Bennett, M. R.; **Autonomic neuromuscular transmission**
Cambridge University Press, London (1972), pp. 274, $ 25.00, £ 7.60

The author of this book, a senior lecturer in physiology at the University of Sydney, has an international reputation in questions related to impulse transmission in the autonomic periphery. In this book, the main emphasis is laid upon the biophysical, physiological and biochemical aspects of transmission. In spite of this, even though structural aspects are dealt with mainly on the basis of light and electron micrographs previously published by others, some of the relevant pictures illustrating nerve–smooth muscle junctions include those of the author (Figs 38—42).

In the first part of the book, structure and electrical properties of the smooth muscle cell are summarized, with special regard to the differences between smooth and skeletal muscles. Since our knowledge on the biophysics of synaptic impulse transmission (resting potential, end plate potential = excitatory post-synaptic potential, quantal release of transmitter substances etc.) is based mainly upon studies performed on neuromuscular junctions in striated muscle, such a comparison is very useful.

In the second chapter, the structure of the junction between autonomic nerve terminal and smooth muscle cell is described and depicted. An important feature is the structural diversity of such nerve–muscle junctions as summarized in the very informative Table 7. In between the two extreme forms, *viz.* the close contact apposition between autonomic axon and smooth muscle cell (resulting in a 200 Å synaptic gap) and the wide intercellular space ("synapse à distance" of the French authors, resulting in distances between innervating and innervated structures up to several micra), there are to be found many intermediate patterns in various muscles of various species. Such differences render extremely difficult the generalization of the biophysical rules of autonomic neuromuscular transmission as attempted in this chapter.

The third part is devoted to the effects of the transmitter released from autonomic nerve terminals. Excitatory and inhibitory junction potentials, spontaneous miniature potentials, quantal release of the transmitter from nerve terminals, duration and termination of transmitter action are discussed in various smooth muscles. Storage and release of the transmitter are illustrated in very instructive block diagrams.

The last part (Chapter 4) gives a brief summary on the neuronal control of smooth muscle. A theoretical explanation is expounded how variations in the number of nerves excited or the frequency of nerve impulse firing controls the contractile force developed by smooth muscle. The bibliography, comprising nearly 500 references, is of considerable importance for scientists working in this field.

Several aspects of autonomic innervation appear to have escaped the attention of the author. Thus, no attempt is made to explain denervation supersensitivity of smooth muscle. Is it due to the spreading of receptors like in denervated skeletal muscle or to some other mechanism? The role of cyclic AMP in the mediation of adrenergic transmission to smooth muscle (Sutherland) and the role of prostaglandins in the regulation of such effects (Clegg) are not included in the appropriate chapters. Even though the author concentrated his efforts in order to present an integrated picture of structural–functional relationships of neurochemical mediation, it is felt that the level of molecular anatomy is still not achieved in the field of autonomic neuromuscular transmission.

B. Csillik (Szeged)

Non-verbal communication

Ed. Hinde, R. A. Cambridge University Press, London, (1972), pp. 443, £ 5.

This book is a comprehensive and well-written account of the proceedings of a study group of the Royal Society on non-verbal communication of animals and man. Inspired by Julian Huxley, this group of psychologists, ethologists and anthropologists decided in a series of 13 meetings to produce papers on their own field of research, but taking into consideration some common principles made clear in the final meeting in September, 1970. The editor, in addition, made serious efforts to synthesize the individual contributions.

The fifteen chapters of this well-edited volume are divided into three sections. The three papers of the first part deal with fundamental problems of the theory of communication, its links with biophysics, linguistics and animal psychology. The second part comprises four articles on animal communication. The papers on communicative behaviour in lower vertebrates and invertebrates, on vocal manifestations in birds and on non-verbal exchange of information in mammals are equally valuable. The third section of the book includes 8 papers of great diversity on "wordless" communication in man. The articles on the phylogeny of laughter, on plays and players and on communication in art are especially worth of interest.

Professor Hinde included introduction and comments in each of the three sections. These editorial notes were circulated among the contributors and were improved in the course of the interchange of ideas. Thus the informative value of the book was increased. This book is essential to those who work in the field of ethology, animal psychology, or human verbal and non-verbal communicative behaviour.

G. Ádám (Budapest)

Principles of receptor physiology
Handbook of Sensory Physiology. Vol. 1. Ed. LOEWENSTEIN, W. R.
Springer Verlag, Berlin—Heidelberg—New York, (1971), pp. 600, DM 168.

The physiology of the receptor apparatus has been considered since PAVLOV and BECHTEREV the clue to the understanding of brain function. Notwithstanding no complete survey of sensory processes has been attempted since BETHE's famous "Handbuch" some fourty years ago. That's why physiologists, psychologists, biophysicists and other scientists in related fields warmly welcome this important and daring enterprise of publishing a complete and authoritative overview of this rapidly developing field of biological knowledge.

The first volume of the series deals with general laws of receptor function beginning from the fundamental principles of bioelectricity in receptor organs to the patterns of organization of sensory systems. It was edited by Professor WERNER LOEWENSTEIN, who contributed to the volume by an excellent paper on mechanoelectric transduction in the Pacinian Corpuscle. The list of the other 18 authors include the most outstanding specialists of the field of sensory physiology and biophysics, among others H. GRUNDFEST, S. STEVENS, M. FUORTES, T. TEORELL, etc. The first chapter on mechano-chemical conversion by the late A. KATCHALSKY and A. OPLATKA, Chapter 10 on mechano-electrical transduction by T. TEORELL and Chapter 14 on sensory transduction in hair cells by A. FLOCK are — among others — the most remarkable contributions of the 19 chapters of the book.

The most useful feature of this volume is its extensive spectrum of topics covering almost all aspects of the general physiology of receptors. The book will be of value not only to specialists in this field, but also to research workers and students in neurophysiology, experimental psychology and behavioural sciences.

G. ÁDÁM (Budapest)

Photochemistry of vision
Handbook of Sensory Physiology. Vol. VII/1. Ed. DARTNALL, H. J. A.
Springer Verlag, Berlin—Heidelberg—New York (1972), pp. 810.

Vision is undoubtedly the most important and thus the most intensively elaborated section of sensory physiology. No wonder that the Editorial Board of this comprehensive series of "Handbooks" decided to publish under the heading of Volume VII four large books on different topics of physiology of vision. The first of this quadruplet deals with photochemical processes. About 80% of the radiant energy emitted by the sun lies in the band of the "visible light", which is an ideal sensory stimulus. Light-receptive cells in the animal kingdom contain photosensitive pigments, which in all visual receptors are built to a common molecular pattern. The common feature of chromoproteins is the guiding principle of this book as emphasized by its Editor in the Preface as well as in his own paper on photosensitivity.

The book contains 18 chapters on different aspects of photochemistry, such as principles of interaction of light and matter (by E. ABRAHAMSON and S. JAPAR), the different functions of pigments (chapters by R. MORTON, by T. YOSHIZAWA, by T. SHOW, *etc.*), the properties of the invertebrate eye (chapters by R. EAKIN, by T. GOLDSMITH, by T. and R. HARA, etc.). All these chapters give the specialist as well as the general reader the picture of how the visual pigments are affected by light.

Photochemistry of Vision reflects the current state of research in this interdisciplinary field. The reviewer of this excellent monograph cannot avoid paying a high compliment to the Editor and to the contributors for the exact and clear presentation of their papers, for the high quality of illustrations and for the wealthy list of references. The book will be of value to students in biophysics, biochemistry, neurophysiology, and even psychology.

G. ÁDÁM (Budapest)

Chemical senses. Part 1. olfaction; part 2. taste
Handbook of Sensory Physiology. Volume 4. Ed. BEIDLER, L. M.
Springer Verlag, Berlin—Heidelberg—New York (1971), pp. 518 and 410.

The fourth volume of the important editorial enterprise Handbook of Sensory Physiology consists actually of two distinct books; one on olfaction including 17 chapters and one on taste physiology comprising 16 chapters. The main feature of both is their phylogenetic attitude;

most of the authors are treating their subject from an evolutionary aspect. This point of view is completely justifiable, since both chemical senses are developed to an optimal level in subhuman species.

The book on olfaction includes many aspects of olfactory function beginning with the comparative anatomy of the nasal structure (by T. S. PARSONS), to the relation of olfaction to ionizing rays (by J. GARCIA and R. KOELLING) and to nutrition (by J. LEMAGNEN). The chapters on neural coding of smell receptors (by R. GESTELAND), on spatial and temporal patterning (by M. MOZELL) and on olfactory theories (by J. DAVIES) are especially worth of interest.

The second part of the volume is a comprehensive monograph on taste physiology, even of higher level of competence than the first part on smell receptors. The chapter on systematics of tongue topography by R. BRADLEY is by itself a remarkable and long-needed contribution. Taste psychophysics is interpreted by C. PFAFFMANN and co-workers, the electrophysiology of taste nerves by Y. ZOTTERMAN, the problem of neural coding by M. SATO. The Editor of the two twin-books has written an excellent paper on taste receptor stimulation with salts and acids.

For those who want an up-to-date survey of the comparative anatomy, physiology, biophysics and psychophysics of chemical senses, this is an excellent and authoritative reference.

G. ÁDÁM (Budapest)

Flora Europaea. Vol. 3
Ed. TUTIN, T. G., HEYWOOD ,V. H., BURGES, N. A., MOORE, D. M., VALENTINE, D. H., WALTERS, S. M. and WEBB, D. A.
Cambridge University Press, London (1972), pp. 370, £ 12, $ 37.50

The third volume of Flora Europaea gives a short complete key to all of the Angiospermae, in volumes 1—3, and the great majority of those will be included in volumes 4 and 5. This volume gives full particulars on the families of Diapensiaceae to Myoporaceae from the Angiospermae. Moreover are in these volumes five Appendices too. In the first appendix are the authors acquainted with the key to the abbreviations of authors' names, the second and third one the periodicals and anonymous works cited in volume 3. Appendix 4 gives a glossary of technical terms, and in the fifth is available a complete vocabularium anglo-latinum, which vocables had been employed above, to the description of species by authors. At the end of the volume are five geographical maps. These show the boundaries of Europe for the purposes of Flora Europea, and its division into "Territories", boundary between Europe and Asia in the Aegean region, and the same in the southern part of the USSR. Maps IV and V are composed to illustrate the meaning to be attached to certain phrases used in summaries of geographical distribution of any taxon. These appendices are followed by an index of families and species.

The third volume of Flora Europaea is a splendid example of international collaboration besides, it can be used by botanists, especially in the European region, but elsewhere in the world too.

J. SZUJKÓ-LACZA (Budapest)

FARKAS, G. L.; **Nucleic acids and proteins in higher plants**
Akadémiai Kiadó, Budapest (1972), pp. 372.

This book of very nice presentation and valuable content presents the results of a Symposium held in the Biological Research Institute at Tihany. It is the first to summarize the international research works concerning the nucleic acids and proteins of higher plants, namely not only from analytical and structural points of view but also the synthesis and hormonal regulation of these substances as well as their role in the metabolism and development are discussed in detail. The volume contains 38 papers classified into five sections (chapters). Its content is characterized by interesting titles: Nucleic acid and protein synthesis in germinating seeds; Methylation of nucleic acids by higher plants; Factors that control RNA polymerase from plant cells; Role of phytochrome in the control of enzyme activity in higher plants; Photomodulation and photodetermination of enzyme synthesis; The role of ATP sulphurylase in the biosynthesis of cysteine in higher plants; RNA synthesis in isolated chloroplasts; Ribosomal RNA synthesis in nuclei of freely suspended cells of higher plants; Sites of synthesis of

chloroplast proteins; Environmental and chemical control of RNA breakdown in leaves; Ribonucleic acid synthesis and hormone action in lentil roots; Nucleic acids and their derivatives in the control of development.

It is indicated even by these randomly selected titles that the book deals with the great, common and actual problems of plant physiology and biochemistry at the highest scientific level. The text is clearly understandable, it does not make any difficulties for the reader. The list of references to each paper, containing mostly even the titles of the papers cited, contributes to an easy orientation.

V. Frenyó (Budapest)

Dickson, J. H.; **Bryophytes of the Pleistocene**
The British Record and its Chorological and Ecological Implications
Cambridge University Press, London (1973), pp. 256. £ 12.80

This book deals with the history of the rich, English bryoflora from the late tertiary till our days.

Author describes in detail the Pleistocene and briefly the neogen-, early-, middle- and late Pleistocene bryoflora of Britain having been uncovered so far. He gives a survey of the localities and literary data in a compact phrasing. This is followed by the description of the present climatic requirements of the species based on its general distribution, then the age of the remains is given using the terminology usual in the palinology and the English stratigraphy. The orientation is facilitated by the excellent map insets. The outline of the present picture of the neogen and pleistocene bryoflora is supported by the world literature. The results of the Polish Palinological school as well as the research works in the Soviet Union are of special importance in that.

The description of the subfossile bryoflora is preceded by a bryogeographical, geological and an extremely detailed plainological survey reflecting the present situation. This survey presents the temporal and stratigraphical distribution of the fossile and subfossile phanerogamous plants, based mainly on results obtained in England. This is the reason why the author, as he mentioned in the preface of the book, relies upon the interest of both plainologists and bryologists. The documentation of the localities and methods of identification presented in the book consolidates the authenticity of the results. The volume is closed with the literature cited and the list of species treated.

J. Szujkó-Lacza (Budapest)

Timofeeff-Ressovsky, N. V., Ivanov, V. I., Korogodin, V. J.; **Die Anwendung des Trefferprinzipes in der Strahlenbiologie**
G. Fischer, Jena (1972) pp. 196, Figs 60, M 29.50

The book is the testimonial of one of the great classics of radiation biology and at the same time a re-evaluation of the application of the target theory.

Within the scope of the general evolutional tendencies of radiation biology authors make the reader acquainted with the history of the origin of the target theory, its exact formulation, application, improvement and its connection with the size of the target as well as the amplification effect. They attempt to apply the target theory to some complex radiobiological phenomena, *e.g.* reparation, cell lethality, tissue regeneration and to find its exact place at the present stage of radiation biology. In the discussion they elucidate a number of misunderstandings and, in addition to the primary processes, take into account environmental conditions, metabolism, etc.

The book translated from the Russian original relies mainly, save a few exceptions, on data published up to 1965 and does not treat the problems at the molecular level. This fact, especially as regards the chapter on reparation, reduces its value. On the other hand, the detailed elaboration of the Russian-language bibliography is filling a gap.

To sum up, the work is a valuable resumé of the classical target theory. Although it is not complete, it calls attention to some suggestive application possibilities.

S. Igali (Budapest)

BEERMANN, W.; **Developmental studies on giant chromosomes**
Springer Verlag, Berlin—Heidelberg—New York, (1972), pp. 227, Figs 110, DM 59.

The book summarizes the knowledge of gene function obtained on the basis of investigations of polytene chromosomes.

The first chapter concerns the structure as well as the relation of structure and function of polytene chromosomes. It involves the quantitative relations of discs and genes within the polytene chromosomes and presents methods for individual and mass isolation of unfixed chromosomes. The book deals with the structural differences detectable between active and inactive genes, the experimental induction of inactive regions of giant chromosomes, as well as with certain steps of gene activation. It summarizes the characteristics of the DNA replication observed on the giant chromosomes of insects.

The processes of transcription are demonstrated on the polytene chromosome of insects and the lampbrush chromosome of the *Triturus* oocyte which are suitable for microscopic investigations and represent good models for studies of transcription. The author points out that although there is a lot of knowledge about the behaviour of the puffs of polytene chromosomes, we are far from understanding of the connections of the phenotype and the control systems. He indicates that further biochemical, physiological and genetical investigations are needed to answer the questions of this field.

The book describes further the connection of differentiation of the organs to the puff formation. According to the author, the results obtained by investigating the puff formation of Drosophila can be extrapolated even to living beings of higher organization, since the experimental puff induction is not specific. He points out the necessity of further studies clearing up the connection between the inductive agent and the reactive gene locus.

After all, the book offers a very valuable summary of the studies on the giant chromosomes and its excellent illustrations well demonstrate them. It offers useful data for the elucidation of the gene functions, however, because of a certain degree of specialization, it can be useful first of all for those working in the field of insect genetics.

IRÉN CSUKÁS-SZATLÓCZKY (Budapest)

RAJKI, S., DÉVAY, M., RAJKI, E.; **Metabolism and heredity, or autumnization as a microevolution**
Agricultural Res. Inst. of the Hungarian Academy of Sciences, Martonvásár (1972), pp. 111

This booklet written in three languages deals with the problem of heredity of acquired properties in connection with the autumnization of spring wheat, evoking many discussions even now. The basic theoretical genetic assumption of the book is not shared by a great part of geneticists and the interest of the book lies just in that.

Authors analyze the periods of historical development of genetics, first of all from the point of view of opinions regarding the inheritability of the acquired properties, then they concern the molecular theory of heredity.

The book offers experimental data for the discussion of question of heredity of acquired properties. The autumnization of spring wheat is used as a model. The metabolic biochemical assumption of the authors in heredity can be regarded as a perspective standpoint. They attempt to describe a detailed experimental programme intending at supporting their opinion that has given rise to much controversy, using the methods of molecular biology. They want to analyze the environmental effects under absolutely standardized circumstances in phytotrone, and the alterations will be followed at the level of macromolecules, first of all of nucleic acids (DNA and RNA synthesis, nucleic acid hybridization, chromosomal analysis, etc.). We are looking forward to the results of this rich molecular programme.

A. TIGYI (Pécs)

POSTGATE, J.; **Biological nitrogen fixation**
Merrow, Watford (1972), pp. 61, £ 1.50

During the recent decade a great advance has been achieved in the elucidation of details of biological nitrogen fixation. Especially the recognition of double nature of nitrogenase proved to be of great significance. It has been revealed that two different protein molecules form a complex in that enzyme stabilized in vivo by a connecting factor. The binding to the

substrate, i.e. the essential function of the enzyme is carried out by two different microelements. All these problems are treated in detail in the text and well constructed schemes of the book.

Since the binding of atmospheric nitrogen, i.e. the biological nitrogen fixation is one of the most important phenomena of the biosphere, it was reasonable to make fundamentals of the modern knowledge accessible for specialists, teachers and students.

The small-sized book of nice presentation contains several good schemes, photographs and electron micrographs.

V. FRENYÓ (Budapest)

Progress in biophysics and molecular biology. Vol. 24
Ed. BUTLER, J. A. and NOBLE, D. Pergamon Press, Oxford—New York (1972), pp. 238, £ 6.50

This volume involves 5 papers. Levine treats in detail the physical methods and the results of investigations of membrane structures. The results have been obtained by X-ray-diffraction, electron spin resonance, nuclear magnetic resonance and spectroscopic investigations. All those are of physicochemical character nevertheless very useful even for biologists. LAND deals with the physics and biology of animal reflectors and describes their structure and function, giving a theoretical basis, too. He lists the different types of reflecting surfaces known in the animal kingdom and describes their characteristics. ZAMYATNIN writes a short chapter on the volume of proteins in solutions. A paper of PALMER and HALL discusses in detail the mitochondrial membrane system, describing its physical and chemical characteristics, morphology, as well as the processes of respiration and phosphorylation: it touches even the biogenesis of mitochondria. The paper of BAKER on the metabolism and transport of Ca-ions in the nerves, presents the own results of the author obtained in invertebrates and excellently reviews the literature. The author assumes, and this seems to be likely, that the role of Ca-ions and the processes connected to them are essentially of universal character and take place similarly even in vertebrates.

The chapters of this valuable volume are completed by abundant and well selected list of references. The presentation of the book is excellent.

G. CSABA (Budapest)

Progress in biophysics and molecular biology. Vol. 25.
Ed. BUTLER, J. A. and NOBLE, D. Pergamon Press, Oxford—New York (1972), pp. 172, £ 4.50

The longest paper in this volume (the paper of J. N. MEHRISHI) concerns the molecular aspects of the cell surface. It demonstrates in detail the method of cell-electrophoresis and the molecular components of the cell surface. It deals in detail and at an up-to-date level with the glycocalyx and describes the phenomena connected to the cell surface in different cells such as tumour cells and normal and pathological lympho- and leucocytes. It covers the problem of radiation injuries and protection from the point of view of the cell surface. This very nice literary review, including the own results of the author as well, is completed by an abundant list of references. The up-to-date level of this chapter is proved at best by the addendum, in which a paper, published in 1972, on the cell surface changes occurring during malignant transformation is discussed.

The short chapter of CANTOR on the T cells (thymus-dependent lymphocytes) offers a good summary of our knowledge and involves even the author's hypothesis. Although it is found elsewhere in the volume, the paper of the other four members of the Mill Hill team on the biosynthesis of immunoglobulins is related to this subject. The similarities and differences of the T and B (bursa-dependent) lymphocytes are also treated in this chapter, offering detailed immunogenetic knowledge. The synthesis and secretion of the polypeptide chains are explained on the basis of this knowledge.

In the remaining part of the volume the paper of BLAKE on the X-ray-diffraction analysis of the crystallized proteins including haemoglobin, proteolytic enzymes, insulin, lysozyme etc. is reviewed.

The presentation of the book, similarly as that of the former volumes, is perfect.

G. CSABA (Budapest)

Progress in biophysics and molecular biology. Vol. 26
Ed. BUTLER, J. A. and NOBLE, D.
Pergamon Press, Oxford—New York (1973), pp. 478, £ 13.

This volume contains 8 papers of very high level. REUTER deals with the divalent cations of the excited membranes involving the problems of all three types of muscle. MCNUTT and WEINSTEIN summarize the latest knowledge on the intercellular connecting structures of mammals, illustrating the text with excellent own schematic pictures and electron micrographs. Apart from the morphological and biochemical data, the functional significance of each structure is elucidated SHA'AFI and GARY-BOBO treat the permeability of the mammalian red blood cell membrane from the point of view of water and non-electrolyte substances. RITCHIE reviews the question of the neural conduction of impulses including the interrelations between heat production, electric activity and metabolism. HUGHES discusses in detail the glucoprotein components of the cell membrane in a biochemical aspect as well as the function of the external coat in cases of different, normal and pathological cells. He also touches upon the simple eukaryotes, bacteria and viruses. The functions of histocompatibility are also treated in detail. HINDLEY's paper is devoted to the structure of phage RNA its replication, translation and gene sequence. The DNA synthesis in prokaryotes is the subject of SMITH's paper, whereas BITENSKY and GORMAN review the literature of the structure and function of the cAMP-adenylcyclase system. The particularly accurate references of the latter paper involve even a part of 1972, the role of cAMP is demonstrated from numerous aspects including even certain clinical points of view.

Each paper is completed by a detailed and up-to-date list of references, and the volume contains also a cumulative index. The presentation of this volume, like that of the previous ones, is excellent.

G. CSABA (Budapest)

CARPENTER, Ph. E.; **Microbiology**
Third edition. Saunders Co., Philadelphia—London—Toronto (1972), pp. 494, £ 4.05

This book is intended for the student who is being introduced to the field of microbiology. This may be the student's only course in microbiology or he may later take advanced specialized courses.

The book is divided into five sections. The first section introduces the student to the study of microorganisms; what they are, how they were studied earlier and how they are studied today.

The second section deals with the biology of bacteria and viruses, their morphology and classification, how bacteria secure energy and building materials, how their growth and death can be controlled. In the third section other microorganisms, viz. protozoa, algae and yeasts are dealt with from similar points of view.

The fourth section introduces the student to the study of diseases caused by microorganisms and viruses. Concepts like infection, virulence, infectious disease, host's resistance and immunity as well as the four principal routes of the transmission of pathogenic agents can be learned from this section. The last section is devoted to environmental and applied microbiology.

The very abundant and instructive illustration and the Tables easy to survey are of great value in this book. The text in general is clear, sometimes too concise to be exact. The nomenclature used (e.g. *Salmonella typhosa* instead of *S. typhi*) and some definitions are not quite up-to date. In the reviewer's opinion the essential difference between bacteria and viruses is insufficiently expressed in this book. Some of the statements are not valid any more, e.g. that typhus fever "is more or less constantly prevalent in Central Europe, Russia and Poland" (p. 133).

In general, the book can be recommended for those who want to be acquainted with the main principles of microbiology without effort.

E. FARKAS (Budapest)

Current topics in microbiology and immunology. Vol. 58
Springer Verlag, Berlin—Heidelberg—New York (1972), pp. 213.

This volume contains five literary reviews. The paper of BOGUSLASKI and co-workers on the applications of bound biopolymers in enzymology and immunology relies on a very abundant literature (more than 500 references). The reader can be acquainted with the application of bound enzymes as membrane models, cytochemical models, models for metabolic cycles, with their use in protein structure studies, in the study of blood clotting, in the analytical field, in the industry, in the therapy etc. The second half of the paper is devoted to the application of bound antigens and antibodies as immunosorbents, immunoindicators, as adjuvants in immunization etc.

BENJAMINI and co-workers review the antigenic determinants of proteins of defined sequences. The amino-acid sequences of 15 biologically important proteins are presented and the related literature is reviewed (more than 200 references) and critically discussed.

The subject of MAJER's paper is virus sensitization, i.e. a specific attachment of antibody to the virion without concomitant neutralization, a phenomenon of significance in some immune-complex diseases.

The remaining two papers (LEVENE; Replication and Lysogeny with Phage 22 in *Salmonella typhimurium* and PHILLIPS; The Morphogenesis of Poliovirus) are recommended for those interested in the highly variable events proceeding in virus-infected cells.

The get-up of the volume is as usual in this series.

E. FARKAS (Budapest)

MICROBIAL COMMUNITIES IN A FOREST—RENDZINA ECOSYSTEM

The pattern of microbial communities

by *I. M. Szabó*

This book provides a deep insight into the community organization and metabolism of the microbiota of a soil ecosystem presenting the results of a large number of analyses and investigations, carried out by using both conventional typing methods and the statistical methods of numerical taxonomy on the composition, changes and fluctuations of bacterial, actinomycete, fungal, yeast, protozoan, etc. populations of diverse plant, animal and soil microhabitats. The author focuses his attention primarily on the fundamental pattern of community organization, the general characteristics of which are independent of the taxonomic composition and of the features and environmental stresses of that particular soil ecosystem in which the community grows and reproduces.

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A kiadásért felel az Akadémiai Kiadó igazgatója. Műszaki szerkesztő: Zacsik Annamária

A kézirat nyomdába érkezett: 1973. IV. 18. — Terjedelem: 11,55 (A/5) ív, 42 ábra (1 színes)

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ACTA Biologica

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TOMUS XXIV
FASCICULI 3–4

AKADÉMIAI KIADÓ
BUDAPEST 1973

ABAHAU 24(3–4) 131–250 (1973)

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A MAGYAR TUDOMÁNYOS AKADÉMIA BIOLÓGIAI KÖZLEMÉNYEI

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Acta biol. Acad. Sci. hung., **24** (3—4), 131—135 (1973)

EFFECT OF 1,6-DIBROMODULCITOL (DBD) ON THE RNA METABOLISM OF S_{37} TUMOUR CELLS

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(Received 1972—04—11)

Abstract

The effect of 1,6-dibromodulcitol (DBD) on S_{37} ascites tumour cells was examined by electron-microscope autoradiography. In mice treated with DBD intraperitoneally, the intracellular distribution of the radioactivity in the tumour cells was changed 120 min after the administration of ^{3}H-uridine, viz. (a) the nucleolar activity was lower, the cytoplasmic activity was higher than in the controls; (b) 48 h after DBD treatment the nuclear radioactive concentration (r. c.) was lower than in the controls; (c) the cytoplasmic r. c. was higher than in the controls, irrespective of the interval (6, 24 or 48 h) between DBD treatment and the injection of the radioactive precursor. It is supposed that some damage in the nuclear membrane or a disturbed metabolism of substances participating in the RNA transport might be responsible for the changes.

Introduction

DBD, a drug widely used in the therapy of human cancer, incorporates into the chromatin of tumour cells and, consequently, counteracts the incorporation of thymidine into DNA and reduces the RNA and protein metabolism of the cells [1, 3—7, 9—11]. In the present work the effect of DBD on the RNA metabolism of S_{37} tumour cells has been studied by electron-microscope autoradiography.

Material and method

C57 Black mice were inoculated intraperitoneally with 5×10^6 S_{37} tumour cells each. Eight days later half of the animals were treated with 300 mg/kg DBD intraperitoneally, the remainder received saline. To examine uridine incorporation, the animals were given 20 μCi ^{3}H-uridine/mouse (SA: 3 330 mCi/mmol per l) 6, 24 or 48 h after the injection of DBD or saline. Thirty and 120 min later peritoneal fluid was sucked off and pooled, using samples from four animals in each pool. The pools were fixed in 2% OsO_4, dehydrated, and embedded in Durcupan. Ultrathin sections were placed on slides coated by celloidin, stained according to KARNOVSKY [8] and covered with Ilford L—4 emulsion [10]. The sections were exposed for 16 weeks at +4°C and developed in Microdol X [10]. The quantitative evaluation was performed as recommended by BERGERON and DROZ [2].

Results

There was no considerable difference in grain count between control and treated animals 30 min after the injection of labelled uridine. In the 120-min preparations, however, a reduced nucleolar activity and an increased cytoplasmic activity were apparent (Figs 1 and 4).

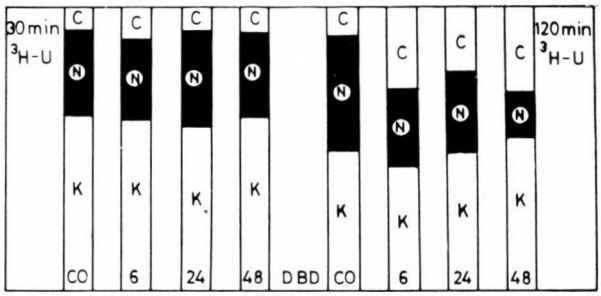

Fig. 1. Grain-count distribution in control and DBD-treated tumour cells at 30 and 120 min after the injection of ^{3}H-uridine. At 30 min there was no difference in grain count of the cytoplasm (C), nucleolus (N) and karyoplasm (K). At 120 min a reduced nucleolar activity and an increased cytoplasmic activity was visible

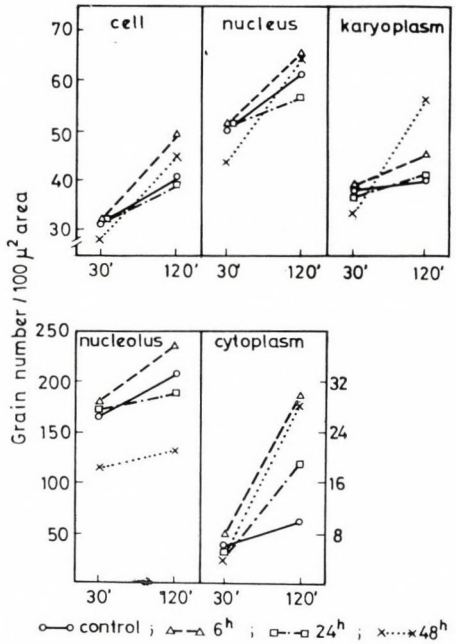

Fig. 2. Changes in radioactive concentrations after DBD treatment. The nucleolar radioactive concentration decreased, the cytoplasmic activity increased 48 h after DBD treatment

The grain count/100 μm^2 (i.e., radioactive concentration) was not influenced substantially by the DBD treatment. A moderate increase in the uridine incorporation was observed at 120 min (Fig. 2).

A reduced nucleolar activity was registered 48 h after DBD treatment (Figs 2 and 4).

Thirty min after the injection of ^{3}H-uridine there was no difference in cytoplasmic activity between control and treated animals. At 120 min, on the other hand, the cytoplasmic activity of the latters was increased, irrespective of the interval between DBD treatment and the injection of labelled uridine (Fig. 2).

The changes in the RNA transport from the nucleolus into the karyoplasm and from the karyoplasm into the cytoplasm are shown in Fig. 3. There

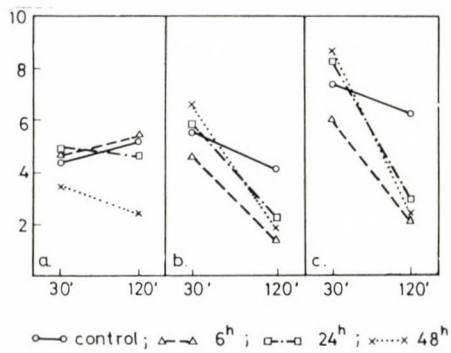

Fig. 3. Changes in ratios of radioactive concentrations at different intervals after DBD treatment

were no differences 30 min after the injection of the precursor, but the differences at 120 min were well-defined: the ratio of nucleolar to karyoplasmic activity showed a decrease 48 h after DBD treatment, due to a decline in the nucleolar activity; at the same time there was a great decrease in the ratios karyoplasmic/cytoplasmic and nuclear/cytoplasmic activity (Figs 3b and 3c), due to an intensive increase in the cytoplasmic activity.

Discussion

The present results suggest that under the effect of DBD the RNA synthesized in the nuclei (nucleoli) is transported into the cytoplasm at an increased rate (the RNA synthesis in the cytoplasm is negligible). This might be attributed either to damages in the nuclear membrane or to a disturbed metabolism of substances participating in RNA transport.

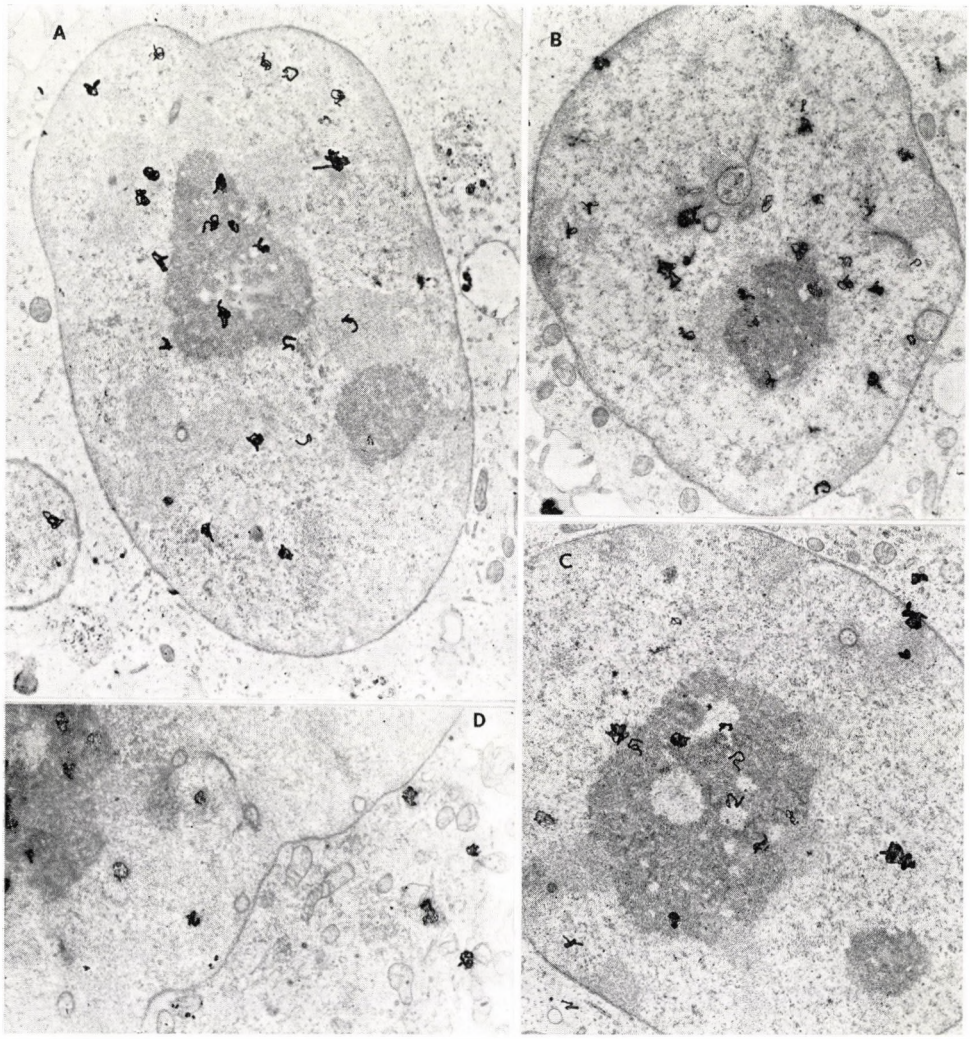

Fig. 4. S_{37} tumour cells: A = control, at 30 min after ^{3}H-uridine injection; B = 24 h DBD treatment, at 120 min after ^{3}H-uridine injection, nucleolar activity; C = 6 h DBD treatment, at 30 min after ^{3}H-uridine injection, karyoplasmic activity; D = 24 h DBD treatment, at 120 min after ^{3}H-uridine injection, cytoplasmic activity

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Acta biol. Acad. Sci. hung. **24**(3—4), 137—156 (1973)

ORIGIN AND BIOLOGICAL ROLE OF MOLECULAR ASYMMETRY

A. S. GARAY, ILONA LACZKÓ, J. CZÉGÉ, K. L. KOVÁCS, L. TOLVAJ, MARGIT G. TÓTH and MARGIT SZABÓ

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(Received 1972—09—10)

Abstract

The molecular asymmetry of living beings is related to the intrinsic asymmetry of matter. In case of $\pi \rightarrow \pi^*$ transitions of structurally similar chiral molecules the intersystem crossing is enhanced by the magnetic transition moment. The phosphorescence which proves the triplet state in $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions is enhanced by the magnetic transition dipole moment. The magnetic transition moment may cause spin-polarization and stereoselectivity in charge-transfer processes. Resonantial energy transfer is enhanced in optically pure systems. The efficiency of photosystem I is roughly proportional to the magnetic transition dipole developed during excitation of chloroplast subunits.

Introduction

No other chemical characteristic is as distinctive of living organisms as optical purity, i.e., the fact that among the two optical isomers only one is present in cells. This asymmetry of metabolites was discovered by PASTEUR in 1848. The decisive experiment was carried out in 1854. "I have kept alive some little seeds (spores) of *Penicillium glaucum* on the surface of ashes and para(-racemic-)tartaric acid, and I have seen that laevo-tartaric acid appears . ." [26]. PASTEUR came to the conclusion that this unexpected stereoselectivity, the exclusive fermentation of the dextro form, reflects the asymmetry of the whole Universe. He writes the following: "Life as manifested to us is a function of the asymmetry of the Universe and of the consequences of this fact. The Universe is asymmetrical . . . Life is dominated by asymmetrical actions. I can even imagine that all living species are primordially in their structure, in their external forms a function of cosmic asymmetry" [26]. He insisted on his statement until his death: starting from his biological points and on the basis of unity of nature he considered matter as asymmetric. Though his opinion has been called an "alchemist dream", it turned out in 1956 that matter really is asymmetric. During the β decay the β^- particles emerging from the nuclei are left-spinning, more precisely they are spinpolarized opposite to their direction of motion. The ratio of spinpolarisation depends on their velocity [21, 42]. This violation of nature's presupposed symmetry was a great sur-

prise because, on the basis of mathematical and physical considerations, most physicists expected the occurrence of the two spins with the same probability. From that time the theory of symmetry-breaking in weak interactions has been elaborated. This discovery has initiated very intensive research. The ideas about symmetries, reflections, invariance principles and even conservation laws have been reinterpreted. At first glance it seems that this field is very far removed from biologists' interests. The speculations of PASTEUR, however, stimulate us to deal with the problem and find the possible links between physical symmetries and life phenomena. This paper, of course, does not presume to reveal the connections, which PASTEUR guessed intuitively, nevertheless it will report about the theoretical and experimental efforts carried out by our group on this topic.

We may perhaps introduce both experiments and speculations with the general theory of reflections. According to LEIBNITZ's principle the mirror image of every object or phenomenon may exist in reality. If we observe the collisions of billiard balls or the movement of a pendulum with an absolutely perfect mirror, we are unable to decide whether we see the events in reality or in the mirror. In spite of this, physicists recognized at the end of the last century that the mirror image of electromagnetic phenomena cannot exist in reality. Let us suppose that an electric current is flowing in a conductor and let us put an ordinary magnetic needle over the conductor. If we reflect this experiment in a plane mirror (the conductor is parallel to the plane !) we see a mirror-image which cannot exist in nature. For, the direction of electric current will not be changed in the mirror, however, the magnetic needle will not follow the well-known right-hand rule. The needle will point in the opposite direction: but it will point in the right direction if the electrons in the reflected current were replaced by positrons. Thus, the exact reflection of electromagnetic phenomena can be carried out only, if by changing right to left, we simultaneously convert matter into antimatter. The above example refers to a plane mirror. In case of β decay the reflection through a centre point is violated in a similar way. In this case we call the interchangeability of left and right parity P symmetry, the interchangeability of charge C symmetry.

After the recognition of parity violation in β decay, physicists were guided by the idea of charge symmetry when they attempted to save the reflection symmetry of nature. They supposed that, if the β^- particles are "left-handed", the positrons emitted during β^+ decay must be "right-handed". Indeed, in the case of ^{58}Co, ^{66}Ga and ^{13}N decay the positrons are left-spinning, they are spin-polarized to the direction of their movement [35]. Until 1964 it was widely accepted that nature can be reflected in a mirror in which both left and right and electrical charges are changed. In that year, however, CHRISTENSON and co-workers [8] proved, that even this complex mirror symmetry is violated. In a high-energy particle accelerator a proton beam was directed at a beryllium

target. The interaction of high-energy protons with neutrons produced many different particles, among them a type of K particles which are CP-symmetrical. Although we cannot go into details here, it turned out that a type of "forbidden" decay did occur, and this is interpreted as breakdown of CP symmetry.

These experimental results were completely unexpected because it was generally accepted that it is impossible to formulate equations of motion without the validity of CP symmetry. According to the present status of the problem, however, the formulation of motion equations postulates only the CPT symmetry and not CP symmetry. This means that the laws of nature can only be reflected in a mirror which, in addition of CP, also reflects the direction of time. It follows from the CPT theorem that the T mirror itself must produce a wrong image in order for the CPT mirror to remain valid [40]. A large number of speculations and experiments based upon this idea are known, but efforts in which T or CPT invariance have been experimentally checked, have failed [28].

We cannot undertake the task to deal with the physical and cosmological consequences of CPT theorem. We need to focus attention only on one particular point, i.e., there is some not fully understood connection between spin, electrical charge and direction of time. The problem is relevant to biology from different points of view. First of all, it must be kept in mind that the "time arrow" has been defined on the basis of thermodynamics and even on the basis of biological considerations [12]. On the other hand, the molecular asymmetry of living beings means that asymmetry appears at a level at which we expect symmetry. Electromagnetic interactions do not violate parity conservation: in other words, no spin direction is privileged. (β decay is not an electromagnetic phenomenon, it occurs due to weak interactions !) The question arises, however, whether any preferential spin direction occurs in interactions of asymmetrical molecules. Instead of speculating, let us omit the problems which are experimentally inaccessible and deal only with the following ones:

(1) Is there any connection between the molecular asymmetry of the cell and the intrinsic asymmetry of matter?
(2) Does molecular chirality have any significance in the energy balance and regulation of metabolism of the cell?

The first question will be reviewed briefly; the second question, however, will be explained in detail.

Material and method

O-, *m*- and *p*-D- and -LD-tyrosine were kindly supplied by Dr. T. HOOKER and Dr. J. SCHELLMANN; authentic L-menthone and D-isomenthone by Dr. J. OSIECKI. We received Mg-deuteroporphyrine from Dr. B. BRIAT. Chlorophyll a and b were prepared by a method described previously [14]. L- and D-DOPA were purchased from National Biochemical Corporation. Details about *Zea mays* mutants and the preparation of chloroplast subunits are found in the papers of FALUDI-DÁNIEL [11, 25].

Tyrosines were dissolved in bidestilled water (2×10^{-5} mol/l) except in low-temperature experiments where an EtOH : MeOH 4 : 1 solvent was used. pH was adjusted by TRIS buffer, low temperature was reached by liquid air.

Absorption spectra were taken on a UNICAM SP—800 spectrophotometer, the CD spectra on JASCO ORD/UV—5 dichrograph. For the luminescence investigation OPTON and HITACHI MPF—2A spectrofluorimeters were used.

The relative quantum efficiency was always measured, but the data for tyrosine were compared with the absolute quantum efficiency determined by others [9, 33].

Stereoselectivity in the chlorophyll—tyrosine system was measured according to [14]. In the system of DOPA-terpenes 0.1% D- and L-DOPA were dissolved in 95% EtOH and the pH adjusted to 9.0. The spontaneous oxidation was followed spectrophotometrically; menthone and isomenthone inhibited the oxidation of L- and D-DOPA, and stereoselectivity was calculated from the rate of inhibition.

The magnetic transition moments were calculated according to MOSCOWITZ [24].

Results and discussion

Origin of molecular asymmetry

The question of the origin of molecular asymmetry in life has attracted a great many speculations and experiments. Especially recently, a large number of reviews have appeared on the topic [4, 18, 38]. The reason of this increasing interest is that the abiogen origin of metabolites has been experimentally proved. In the simulated atmosphere of the primitive Earth different kinds of radiation and electrical discharges gave rise to amino acids, sugars, nucleotides, etc. All of these metabolites, however, occur in a racemic mixture [29]. According to most theories, optical purity developed step by step during evolution. The fact that L-amino acids are present instead of D isomers is supposed to have been caused by chance. Nevertheless, there are two theories, according to which the molecular asymmetry of living beings is related to the structure of matter itself, that is, it is a reflection of the intrinsic asymmetry of matter. VESTER [36], as early as 1957, has directed attention to the parallel between the asymmetry of matter and the optical asymmetry of metabolites. In fact, it has been known for a long time that optically active substances can be synthesized in circularly polarized light, because the two enantiomers absorb different amounts from left resp. right circularly polarized light. But what is the source of circularly polarized light in nature? It has been shown that when the electrons emitted in β^- decay slow down, they loose some of their energy by emitting γ radiation, and this so-called "Bremsestrahlung" is left-circularly polarized. VESTER and co-workers [37] carried out experiments on this basis but obtained no unequivocal results. One of us, using a similar hypothesis, started to work in 1961. After many unsuccessful experiments, D and L isomers of tyrosine were bombarded with β^- particles. The D isomer decomposed more quickly [13]. This result proves that, if during the chemical evolution both isomers were synthesized, β^- decay initiated the selection of one of them. According to CALVIN [7], at the "primordial" Earth 20% of the energy on sur-

face derived from β^- decay. It is of course possible, that β^- particles of circularly polarized γ radiation might have served as energy source for abiotic organic syntheses! HASSELSTROM and co-workers [19] were able to synthesize glycine, aspartic acid and diamino-succinic acid by bombarding $(NH_4)_2CO_3$, with β^- particles from a van de Graaf accelerator. In another experiment γ-rays from ^{60}Co proved to be effective in synthesis of organic molecules [17].

One comment must be made; though the induction of optical activity by polarized β rays has been proved, we think that other mechanisms also took part in the further evolution of optical purity. It has been shown that chemical reactions are not the same in optically pure systems and in racemic mixtures. If differential equations for the kinetics of two mixed enantiomeric populations are solved, no stable dynamical equilibrium is found except when one of the two populations has been extinguished [1, 30]. We think, however, that the advantage which one enantiomer has gained over the other is in the last resort due to parity non conservation in weak interactions!

Another hypothetical connection between parity violation and the asymmetric appearance of biomolecules on Earth has been suggested by YAMAGATA [43]. He postulates a sequence of n reactions leading to the real (D-deoxyribose containing) DNA. For the synthesis of the mirror-symmetrical "imaginary" (containing L-deoxyribose) DNA the same number of reactions is needed. Now, because of the breakdown of the parity principle in weak interaction the reaction rate is not exactly the same in the case of the real and imaginary DNA. The theoretically expected difference is very small, it is of the same order of magnitude as the coupling constant in weak interactions. If the coupling constant of electromagnetic interactions is 1/137, then the coupling constant of weak interactions equals $10^{-6}-10^{-7}$. The reaction rate of one step in the synthesis of real DNA molecules would therefore be $1 + 10^{-6}-1+10^{-7}$ if the reaction rate of the corresponding step in the synthesis of an imaginary molecule were the chosen unity. YAMAGATA defines p_k as the ratio of reaction rates for the kth reaction in the real sequence relative to that in the imaginary sequence. For simplicity let us assume that all $p_k = p$. Then

$$\frac{N_v}{N_i} = p_1\, p_2\, p_3 \cdots p_n = p^n$$

$$p^n = (1 + \varepsilon)^n \approx e^{\varepsilon n}$$

where ε is the coupling constant of weak interactions: $10^{-6}-10^{-7}$, N_v and N_i stand for the polymerization rate of real and imaginary DNA. Because the number of nucleotides in DNA molecules in a cell is supposed to be 10^8-10^9, therefore

$$\frac{N_v}{N_i}\ e^{\,10^{-6}\times 10^{}}$$

This fascinating result means that in case of a sufficiently great number of reactions the small differences will accumulate and lead to the complete prevalence of the real polymer [see also 4]. Recently THIEMANN and WAGENER [34] have published a remotely supporting observation of YAMAGATA's hypothesis. According to their observations the lattice energies of the enantiomorphic crystals must differ, and this difference can result only from an asymmetric contribution to the interaction.

As BONNER [4] says, the connection between fall of parity and optical activity certainly merits critical duplication and expansion.

Significance of molecular chirality in the regulation of metabolism

In the introduction we mentioned the experiment of PASTEUR with *Penicillium glaucum*. Since that time a large number of similar experimental results have accumulated. We know that the effects of drugs and hormones depend on their chirality, or at least that one enantiomer is much more active than the other. No far-reaching consequences similar to those of PASTEUR however, have been drawn from this type of experiment. Stereoselectivity, i.e., the sorting out of one enantiomer by the cell has been attributed to geometrical causes. This is quite obvious for a biologist accustomed to chemical formulae and sterical models of molecules. We do not wish to deny the relations between the geometry of smaller molecules and the conformation of polymers. The entire geometry of the structure of DNA depends upon the arrangement of groups about asymmetric carbon atoms of deoxypentose. A base-paired helix would be quite impossible if half the molecules of 2-D-deoxyribose had been replaced by 2-L-deoxyribose, because the 1', 3' and 4' asymmetric carbon atoms are involved in forming chemical bonds. In the case of polypeptide the situation is not so simple. A polypeptide can be composed of a mixture of L- and D-amino acids, but the DL polymers propagate much more slowly than the L and D forms. In experiments with γ-benzyl-glutamate-N-carboxy-anhydride the DL mixture polymerized at only one twentieth the rate of either the L- or D-anhydride. According to [3], this very great difference cannot be explained on the basis of the molecular geometry. We may reasonably assume that something happens between the collisions of reactants and the separation of products which cannot be ascribed to the static geometry of the molecule. On the following pages we wish to elucidate the kinds of special phenomena which occur during the excitation of chiral molecules, and what are the intra-, and intermolecular consequences of these phenomena. We hope that on this basis we can answer the question of why certain reaction-paths are preferable to others, and why some processes are faster than others [27]. With this we reach the submolecular, electron level of the activity and regulation of biological systems.

The intramolecular consequences of chirality

If a molecule absorbs a photon, the electron orbitals will be rearranged, and consequently an electric transition dipole moment is induced. In chiral molecules, the static charges are helically distributed. Therefore the helical redistribution of charge during an electronic transition corresponds to a collinear electric and magnetic transition dipole moment, parallel for the right-handed and antiparallel for the left-handed helix. The parallel moments exhibit a

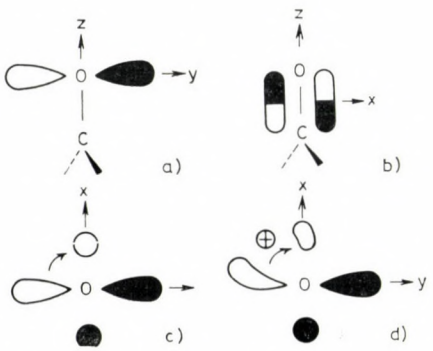

Fig. 1. ***n*** and π orbitals in the carbonyl group and the distortion of orbitals by a substituent having + charge (after [5])

positive Cotton effect, the antiparallel a negative one [6, 23]. It should be noted that the different transitions of the same molecule may be of different helicity. This may be easily understood if one keeps in mind that the electric dipole transition moment does not point toward the same direction in case of different transitions, while the asymmetric distribution of perturbing charges always remains essentially the same.

Let us show, by an example, how substituents can make carbonyl $n \rightarrow \pi^*$ transition optically active [5]. Fig. 1/a symbolizes the nonbonding (n) orbital, Fig. 1/b the antibonding orbital of the carbonyl group. These orbitals are perpendicular and so do not overlap (1/c); in other words, the transition of an electron from the n orbital to the antibonding π orbital is forbidden. The deformation of the orbital system may permit the transition to some extent and thus we get a weak absorption band at about 290 nm. Let us now imagine that a positively charged substituent is fixed near the system. That will of course distort the symmetrical orbitals and thus during the $n \rightarrow \pi^*$ transition the orbitals of the carbonyl group will be rearranged with a right-handed helicity and will produce a positive Cotton effect. In other words, a collinear and magnetic dipole transition moment occurs.

Now the question arises what are the consequences of this magnetic moment on life processes, such as the interaction of excited molecules or electron

transport? At first glance, the question seems to be unrealistic, because the magnetic transition dipole is very small, it is about 10^{-18}cgs. One can, however, point out that the strength of the magnetic field at a distance of 0.1 nm from the chromophore may reach about 1 000 Gauss. This magnetic field is sufficiently strong thus the magnetic moment of the electron may interact with the magnetic transition dipole. In this way the probability of spinflip and consequently the probability of triplet state will be nonvanishing. For the

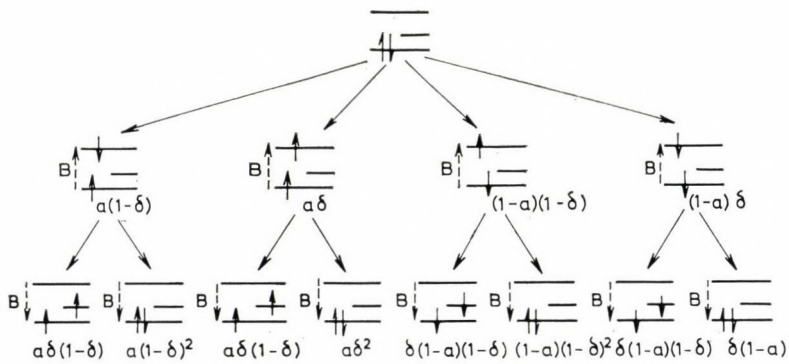

Fig. 2. The possible electronic states in chiral molecules (ground, singlet and triplet)

process responsible for the population of triplet state, the so-called intersystem crossing, is a spin-dependent internal conversion. Let us take as a first approximation the simple model of three electronic energy levels. In Fig. 2 we have indicated every possible transition into singlet and triplet states — except the occurring of the triplet state from the ground state by direct absorption. The possibility of falling back into the ground state is also indicated. $\delta = \delta(B)$ stands for the probability of the spin flip; $a = a(B)$ is the probability that the absorption of a quantum by a molecule raises that electron to a higher electronic level which has a spin magnetic moment opposite to the direction of the magnetic field induced in the molecule; $W(B)$ is the probability of the triplet state in the presence of the magnetic induction B. Investigating all possibilities,

$$W(B) = a(1-\delta)\delta + a\delta(1-\delta) + (1-a)(1-\delta)\delta + (1-a)\delta(1-\delta) = \\ = 2\delta(1-\delta)$$

Therefore, the occurrence of triplet state will depend on $\vec{B}$ (Fig. 3). Because δ increases with B the magnetic field occurring during excitation increases the probability of the triplet level [16].

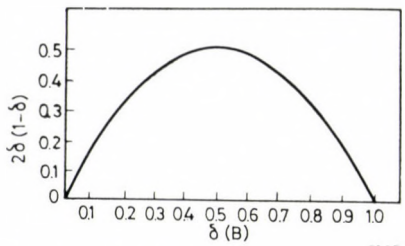

Fig. 3. The probability of the triplet state in chiral molecules as affected by the magnetic induction during excitation

One comment must be made. According to the present theory it cannot be said that the electron having α or β spin is excited because the singlet state is the linear combination of two states:

$$\frac{1}{\sqrt{2}}(\Psi_{\alpha\to\alpha} - \Psi_{\beta\to\beta})$$

In spite of this, we think that (by introducing a) in chiral molecules the idea of linear combination cannot be applied without restrictions because of the magnetic transition moment. The question of how magnetic dipole may influence the occurrence of molecules in triplet state has been investigated experimentally. We have determined the magnetic transition moment of mole-

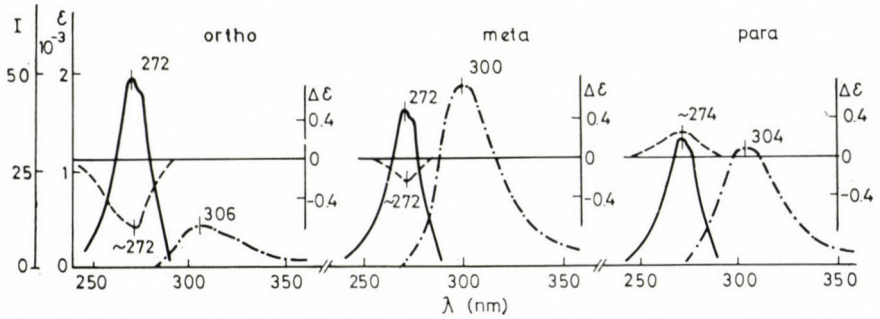

Fig. 4. Absorption (———), CD (— — —) and fluorescence (— . — . —) spectra of *o*-, *m*-*p*-tyrosine

cules which have similar structure by means of absorption and circular dichroism measurements, and have studied whether the population of the molecule in triplet state is increased if the magnetic transition dipole is greater. The measurements of luminescence indicated the presence of triplet state. For it is known that, when a molecule changes from the excited singlet state into the ground state, one part of its energy is emitted in the form of fluorescent light.

The wavelength of this emitted light is always longer than that of the absorbed light, and the absorption and emission spectra — in simple cases — are approximately mirror-symmetrical. The mechanism for intersystem crossing involves both spinflip and vibrational coupling between the excited singlet state and triplet state. Therefore, intersystem crossing competes with fluorescence emission, and if the molecule falls back to the ground state from a triplet state, the quantum efficiency of fluorescence will be decreased, and phosphorescence appears. This has longer wavelength and longer decay time. It is clear from the above that the quantum efficiency of fluorescence must decrease with the increase of the magnetic transition moment, but the quantum efficiency of phosphorescence runs parallel with the increase of magnetic transition dipole.

In the first set of experiments a $\pi \rightarrow \pi^*$ transition has been studied. The $^1L_b(A_{1g} \rightarrow B_{2u})$ transition of *o*-, *m*-, and *p*-D-tyrosine has been chosen [20]. Fig. 4 and Table 1 show the results. One may see that in accordance with our expectations, the quantum yield of fluorescence changes inversely with the increase of the magnetic transition moment: the quantum yield is small if the magnetic moment is great and *vice versa*.

Table 1

Electric and magnetic transition dipole moments and the quantum yield of fluorescence in o-, m-, and p-tyrosine
($\mu_e(D)$ = electric transition dipole moments in Debye units; $\mu_m(\mu_B)$ = magnetic transition dipole moments in Bohr magnetons; Q = absolute quantum yield of fluorescence).

Material	$\mu_e(D)$	$\mu_m(\mu_B)$	Q
o-tyrosine	1.235	0.0062	0.12
m-tyrosine	1.136	0.0021	0.27
p-tyrosine	0.881	0.0039	0.21

Though these results are in line with our expectations, they do not inform us about the population of the triplet state. The only conclusion we may draw is that in case of greater magnetic transition moment the excitation energy is used for fluorescence with smaller probability and it is dissipated in the vibrational levels of singlet or ground states. Another point is that the structure of molecules is not exactly the same and the small differences may influence the quantum yield of luminescence.

For this reason we carried out experiments in which the magnetic moment of the *same molecule* D-*p*-tyrosine was determined in water solutions of different pH-s. As Fig. 5 shows, the magnetic transition dipole increased, whereas the quantum yield of fluorescence decreased as a function of pH, in

particular at low values of pH. These results agree well with earlier findings [9, 33].

Phosphorescence, characteristic of the triplet state, cannot be observed at room temperature in case of $\pi \rightarrow \pi^*$ transition. Therefore, experiments were carried out at low temperature. The results are represented in Table 2. Due to the special — non-freezing — solvent, the CD signal was very small even

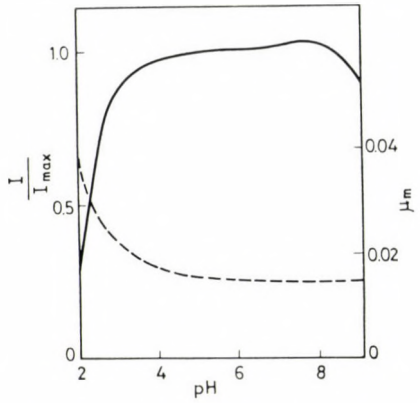

Fig. 5. Effect of pH on the relative quantum efficiency of fluorescence (———), and on the magnetic transition moment of tyrosine (— — —)

at low pH, thus we could not calculate the magnetic moment. At 130°K the magnetic transition dipole was considerably higher and phosphorescence appeared at 410 nm. The intensity of the phosphorescence in relation to the fluorescence increased parallel with the magnetic moment. This proves that the population of the triplet state is increased by increasing magnetic transition dipole.

Table 2

Effect of temperature on magnetic transition dipole moment and on luminescence of tyrosine
(f_q = intensity of fluorescence, p_q = intensity of phosphorescence)

Material*	T(°K)	$\mu_m(\mu_B)$	$\frac{p_q(400\text{ nm})}{f_q(310\text{ nm})}$
Tyrosine	130	0.042	0.35
pH 6.5	298	0.001	0.00
Tyrosine · HCl	130	0.069	1.23
pH 3.5	298	0.001	0.00

* Solvent EtOH : MeOH 4 : 1

One may object that the results which we obtained at low temperature are irrelevant from the biological point of view. Concerning this it should be kept in mind that intersystem crossing competes with fluorescence even at room temperature. Thus the triplet state is populated but, because of the high temperature, the excitation energy is transferred mostly by collisions. Therefore, phosphorescence can be observed only very rarely in liquid media. If the

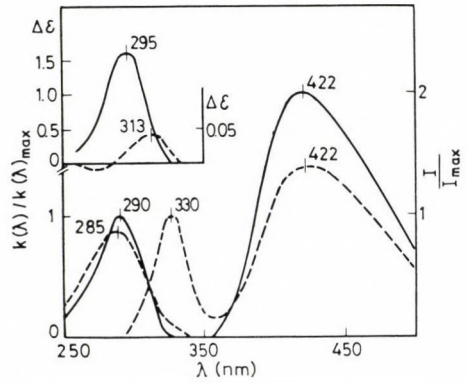

Fig. 6. Absorption, CD and luminescence spectra of menthone (— — — —) and isomenthone (————). (The CD scale on the right side corresponds to menthone)

number of collisions is considerably decreased by low temperature, phosphorescence becomes observable.

In another series of experiments the $n \rightarrow \pi^*$ excitation of the carbonyl group of menthone and isomenthone was studied. In this type of transition the luminescence can be observed only with highly sensitive instruments. This is, of course, a disadvantage. On the other side, it is an advantage that the singlet triplet split for n, π^* states is smaller which tends to enhance intersystem crossing. This accounts for the fact that intersystem crossing occurs with much greater probability from an n,π^* excited state than from a π, π^* state, therefore, in the former case phosphorescence can often be observed at room temperature.

Menthone and isomenthone are stereoisomeric terpenes. In menthone the equatorially oriented isopropyl group is situated roughly in a nodal plane and thus causes only a small positive circular dichroism. In isomenthone, however, the isopropyl group, axially oriented, is situated on the right side of the carbonyl, thus increasing substantially the positive circular dichroism of the $n \rightarrow \pi^*$ transition of the ketone [39]. Fig. 6 shows the absorption, CD and luminescence spectra. The luminescence spectrum of menthone shows two bands, the typical fluorescence band at 330 nm and the phosphorescence band at 420 nm. In case of isomenthone only the phosphorescence band at 420 nm

is present. The disappearance of the luminescence band and the positive correlation between magnetic transition dipole and phosphorescence provide strong support for the above hypothesis. It is remarkable that menthone has a double CD signal. We think that this indicates a mixed state, however, this is irrelevant with respect to the problem discussed here.

The electric and magnetic dipole moments, the relative quantum yield of fluorescence and phosphorescence were calculated from the spectra. As Table 3 shows, increasing magnetic moment enhances the population of triplet state even in case of $n \rightarrow \pi^*$ transitions.

Table 3

Electric and magnetic dipole moments and the relative quantum yield of fluorescence and phosphorescence in case of menthone and isomenthone
($Q_{f_{rel}}$ = relative quantum yield of fluorescence;
$Q_{r_{rel}}$ = relative quantum yield of phosphorescence)

Material	$\mu_e(D)$	$\mu_m(\mu_B)$	$Q_{f_{rel}}$	$Q_{p_{rel}}$
Menthone	0.312	0.0032	1.00	0.72
Isomenthone	0.307	0.1490	0.00	1.00

The intermolecular consequences of chirality

From the foregoing discussion it is clear that molecular chirality facilitates intersystem crossing, and thus chiral molecules easily appear in triplet state. It is known, however, that the reactivity of the molecule is considerably increased in triplet state. For, the excited electron, and the one left behind in the orbit from which it was excited, are no longer paired. Any other unpaired electron-attacking agent may now form a bond, as soon as its orbit begins to overlap with those of the excited molecule, and the potential barrier to reaction is thus lowered. In addition, the excited electron itself is in a more weakly bound orbit often extending over a larger region of space than the orbit from which it was excited. This electron is thus more likely to be removed by an electrophilic reagent [27].

These consequences of the triplet state in reaction kinetics are well known. From a biological point of view, however, another question arises: is it of any significance whether the excitation into the triplet state occurs in racemic or in optically pure solutions? Let us first investigate this problem theoretically. It has been shown that the magnetic moment of the electron interacts with the magnetic transition moment, consequently, among the three possible triplet states one will preferentially come into existence. In other words, molecular chirality will facilitate a triplet state in which the spin-magnetic moments are antiparallel to the magnetic transitional moment. Therefore, the spin direction

of the unpaired electrons in the two enantiomers must be opposite. The situation is represented in Fig. 7. In electronic transitions showing positive Cotton effect, the B state, in case of negative Cotton effect the A state is preferred. Figure 7 shows the direction of the electric dipole moment. It should be recalled that in case of positive Cotton effect the magnetic dipole is parallel to

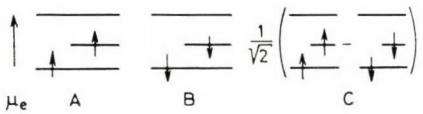

Fig. 7. Diagrams of the three possible triplet states

it; in case of negative Cotton effect the electric and magnetic transitional moments are antiparallel. It follows from these considerations that in optically pure systems at least partial spin polarization may occur, which may be of significance both in electron transport and other types of energy transfer.

We come to a similar conclusion if not the triplet state, but the ionization of chiral molecules in optically pure systems is considered. If, during ionization, a chiral molecule loses its electron, the asymmetrically distributed static charges will influence the "path" of this electron. Therefore, a magnetic moment will be induced in the same way as described above. Thus, in the reduction of chiral electron-donor molecules the average spin of the lost electrons will be different from zero, in other words, spin polarization may occur. Similarly, the uptake of polarized electrons by a chiral electron acceptor will depend *inter alia* on its magnetic transitional moment induced during excitation or ionization. Therefore, we may expect differences if charge transfer takes place between L-L, respectively D-L molecules. In other words, discrimination capacity and stereoselectivity can be traced back to the magnetic transitional dipole of chiral molecules. Geometrical factors undoubtedly may play an important role also, but in addition to them the phenomena outlined above should be taken into consideration.

Optical purity and electron transport

Two experiments were carried out which were concerned with stereoselectivity in electron transfer. In the first, three different Mg-porphyrines were chosen as electron donors. Mg-deuteroporphyrine is not chiral; no magnetic moment is induced in it during excitation or ionization. Chlorophyll a and chlorophyll b are chiral; during excitation into the first singlet the magnetic dipole is smaller in case of chlorophyll a than chlorophyll b [14]. Chlorophylls may easily loose one electron in alkaline medium, which is the first step of allomerization [31]. Tyrosine is able to take up the lost electron reversibly

from chlorophyll, and this way the decay of chlorophyll is inhibited [2]. Thus, chlorophyll $\overset{e^-}{\rightleftharpoons}$ tyrosine are members of an electron-transfer system; the question arises whether L-tyrosine works in this system as well as D. Fig. 8 shows that tyrosine inhibits the allomerization of chlorophylls. Figure 8 and Table 4 show that the speed of allomerization is qualitatively proportional to the

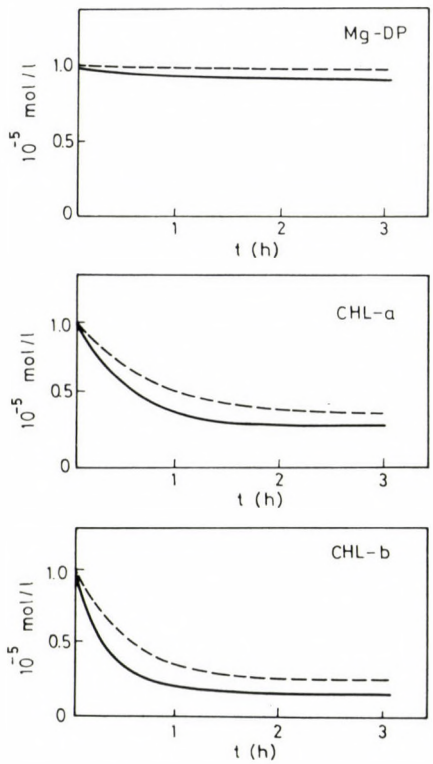

Fig. 8. Decomposition of Mg—DP, chlorophyll a, chlorophyll b in buffer solution (————) and in the presence of 1.48×10^{-3} mol/l LD-tyrosine (— — —)

magnetic transition dipole of the first singlet state. Table 4 also yields information about stereoselectivity. As one may see, only the chorophyll-b $\overset{e^-}{\rightleftharpoons}$ tyrosine electron-transfer system exhibited a weak stereoselectivity in full accordance with the fact that chlorophyll b reveals the greatest magnetic transition moment.

In the second experiment L- and D-DOPA served as electron donors and they were brought together with electron acceptors of different chirality, i.e., with L-menthone and D-isomenthone. Stereoselectivity was surprisingly great, as shown by Table 4. The connection between stereoselectivity and the magnetic transition dipole of the first singlet is conspicious even in this case.

Table 4

Stereoselectivity in the electron-transfer system of chlorophyll + tyrosine and DOPA + terpens (D/L = ratio of stereoselectivity)

Material	$\mu_e(D)$	$\mu_m(\mu_B)$	D/L
Chlorophyll-a	4.883	0.024	0.50/0.50
Chlorophyll-b	4.040	0.041	0.47/0.53
Mg—DP	0.875	0.000	0.50/0.50
Menthone	0.312	0.003	0.58/0.42
Isomenthone	0.307	0.149	0.74/0.26

Optical purity and energy transfer by resonance

The question whether optical purity may influence resonantial energy transfer was studied by concentration quenching. It is known that the quantum yield of fluorescence in solutions depends on the concentration of the fluorescing molecule. In highly diluted solutions (10^{-6}—10^{-5} mol/l)the intensity of the fluorescence parallels the concentration. After reaching a certain concentration level the intensity of fluorescence remains the same; if concentration is further increased, the fluorescence intensity starts to drop. This is called concentration quenching, and is explained by the association of molecules in highly concentrated solutions. The excited molecule forms a dimer with a ground-state molecule of the same species.

$$A + A^* \rightarrow (AA)^*$$

Because of the fact that in the $(AA)^*$ dimer — often called excimer — different kinds of energy-transfer processes may occur, the emission of fluorescent light will decrease. Among the strongest support for this hypothesis is the fact that the fluorescence-light emission increases if the dimers are separated by a slightly higher temperature [22]. Though the theory has proven to be very useful, it

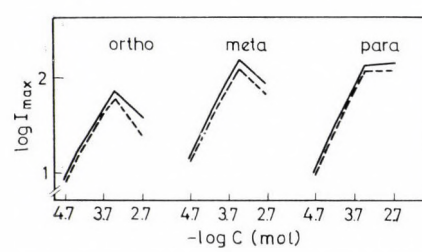

Fig. 9. The concentration quenching of fluorescence of *o*-, *m*-, and *p*-tyrosine in a racemic mixture (———) and in an optically pure solution (— — —)

was unable to explain why the life time of the excited state decreases with increased concentration. One has to suppose that the excitation energy can be transfered from one molecule to the other without light emission by resonance. On the basis of the above statement we have investigated the concentration quenching of *o*-, *m*-, and *p*-tyrosine in optically pure solution containing only D isomers and in racemic mixtures. As Fig. 9 shows, the energy transfer is advanced in optically pure solution.

Magnetic transition moments in highly-organized biological systems

In the previous chapters small molecules have been considered to which a biological function can hardly be attributed. It is of importance to investigate also at least one *in vivo* system. From normal and carotenoid-deficient mutants chloroplast subunits were prepared in which photosystem I remained uninjured [15] yet were suitable for investigation with optical methods. In the subunits prepared from the mutants both the photooxidation of P_{700} and

Table 5

Electric and magnetic transition dipoles, quantum yield of the photooxidation of P_{700} and cyclic photophosphorylation in normal and mutant chloroplast subunits (photosystem I)

Material	μ_e ($10^{-3}D$)	μ_m (μ_B)	$h\nu/P_{700}ox$**	ATP* cyclic
Normal	0.165	0.11	0.50	1.9
Lycopenic	0.153	0.05	0.20	1.5
ϱ-carotenic	0.170	0.07	0.26	1.0

* Mol/mol chlorophyll minute [25]
** [11]

cyclic phosphorylation are of less efficiency [25]. Table 5 shows the quantitative data and enables a comparison between them and the magnetic moments induced in subunits. For the sake of simplicity, in calculating the magnetic moment, both absorption and CD signal of the subunits in the red region were considered to have been originated from a single chromophore. Table 5 shows that a higher magnetic moment is accompanied by increased biological activity when this simplification is introduced. One comment should be made: cyclic phosphorylation which has been measured in non-fractional chloroplasts is influenced also by photosystem II. The independence of photosystem II from the photooxidation of P_{700} may explain the fact that the quantum efficiency of the photooxidation of P_{700} and the cyclic phosphorylation are not fully parallel.

The magnetic moment occurring during excitation and the ATP production are on two opposite ends of a long metabolic pathway. The possible connection between them must be elucidated in future work. The possible spin-polarization in photosynthetic charge-transfer reactions, however, cannot be left out of account. If the transported electrons are considered as an electron gas, then the energy increase — compared to unpolarized electron transfer — can be calculated by Fermi statistics. One may object that this is a very rough approach, as the transported electrons in biological systems are not free electrons. We nevertheless use this approach because no others are at our disposal. According to generally accepted data, the volume of an average chloroplast equals 33×10^{-12} cm^3, in which 2×10^9 electron-donor chlorophyll molecules are present. Under these conditions the energy difference between polarized and unpolarized states is 391.0 cal/mol. Though this value may change by changing either electron density or temperature it gives information concerning the order of magnitude.

Despite the recognition of the importance of electron-biology, nobody, to our knowledge, has studied the biological significance of the magnetic properties of electrons. The idea of spin-polarization or the predominance of a specific triplet state could not have presented itself when the theory of molecular chirality was in an underdeveloped state, and when proper instruments were not yet available. There is every reason to hope that an up-to-date theory of chirality may lead to the solution of the problem why strange enantiomers occur in physiologically important materials and processes, such as in the cell wall of bacteria, in antibiotics, during insect metamorphosis, in stimulus transfer in nervous systems, or — which is a controversial question — in neoplastic tissues. Nevertheless, it seems that electron spin, magnetic transition moments or specific triplet states may serve as signals in regulatory mechanisms; they may influence reaction rates and direction, diminish activation energy or facilitate a better energy balance.

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Acta biol. Acad. Sci. hung., **24** (3—4), 157—163 (1973)

THE CHLORAGOSOMES OF LUMBRICIDAE AS CATION EXCHANGERS

IN VITRO INVESTIGATIONS

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(Received: 1973—02—26)

Abstract

The chloragosomes of Lumbricidae are water-insoluble polyelectrolytes containing active groups able for acid dissociation. Their role in cation exchange has been proved *in vitro*. The possible physiological functions originating in the cation exchange character are discussed. The cations of chloragosomes can bind a considerable amount of anions by means of adsorption.

Introduction

Among the acridine derivatives trypaflavine (acriflavine) and atebrin possess a considerable toxic effect on living organisms, if they are linked to nucleic acids [1—3, 18] or intercalated [15]. Trypaflavine has an intense affinity even to other biological substances of acid character, e.g., sulphated mucopolysaccharides [12, 19, 24] and cerebroside-sulphates [10].

We have found trypaflavine and atebrin displaying a relatively low toxicity in the organisms of Lumbricidae [7] and the majority of acridine derivatives injected in the body cavity bound to chloragosomes [8]. These results prompted us to study the trypaflavine-binding mechanism of chloragosomes. It has turned out that the cation character of trypaflavine plays an essential role in the binding mechanism and it is exchangeable with counterions. Therefore, our investigations were extended to the binding-mechanisms of other cations and anions, too.

Material and method

The experiments were carried out *in vitro* first of all on isolated chloragosomes of *Lumbricus terrestris* and *Allolobophora caliginosa*. Chloragosomes were isolated by means of a modified Urich's method [12, 20], then washed two times with bidistilled water, distilled in a glass apparatus.

Investigations on binding and release of trypaflavine. A 100 mg samples of chloragosomes was suspended in 5—10 ml 0.005% trypaflavine (Hoechst AG) solution, allowed to stand for 10 min and centrifuged. The trypaflavine in the supernatant was measured in a spectrophotometer type Spektromom 361 at a wave length of 650 nm. For comparison, the trypaflavine uptake by a homogenate of 100 mg body wall was measured by the same method.

To characterize binding capacity, the distribution quotient

$$\frac{\text{weight of ions in the internal solution (bound)}}{\text{weight of ions in the external solution (free)}}$$

was calculated.

In order to investigate the release of trypaflavine, the treated chloragosomes were washed several times in 5 ml water, then with 1% $CaCl_2$ until the amount of released trypaflavine strongly decreased. The extinction of the washing solutions was measured at 650 nm. The trypaflavine release of the body-wall homogenate was similarly tested. Apart from $CaCl_2$, 1% NaCl and 1% histidine were applied as washing fluids. Binding to and release from chloragosomes of atebrin (Bayer) and thionine (C. I. 52 000) were also investigated, however, photometric measurements and numerical evaluation have not been carried out.

Measurement of H^+—Ca^{++} ion exchange of chloragosomes. A 100 mg sample of chloragosomes was suspended in 5—10 ml 0.1 N-HCl, allowed to stand for 10 min and centrifuged. They were washed subsequently with water and, at last, 4 or 5 times with 1% $CaCl_2$ at 5 min intervals. The pH of the supernatant was measured with a Radelkis OP 105 pH meter.

To investigate the H^+ — trypaflavine ion exchange, a similar procedure was carried out, except that the preparation was washed with a 0.01% trypaflavine solution instead of $CaCl_2$.

Potentiometric titration of chloragosomes [11]. A chloragosome fraction of 100 mg transformed into the H^+ form (i.e. treated with HCl) and washed with water until acid-free, was suspended in 5 ml 1 mol/l NaCl solution, then titrated with 0.01 N-NaOH, using a glass-calomel electrode. The NaOH solution was added by 0.1 ml volumes and the pH value was registered 10 min later. The pH values were plotted against the uptake of the added NaOH.

Binding and release of eosin. A 100 mg sample of isolated chloragosomes was suspended in 5 ml 0.01% eosin (water-soluble, Chroma), allowed to stand for 10 min and centrifuged. The eosin content of the supernatant was measured at 900 nm. For comparison, the eosin uptake of 100 mg body-wall homogenate was measured. Distribution quotient was calculated as described above. To measure eosin release, eosin-treated chloragosomes were washed with water, 1% $CaCl_2$ of pH 5, 1% $CaCl_2$ of pH 8 and 0.1 N-NaOH. Each washing solution was 5 ml in volume. Extinction values of the supernatants were measured.

OH^-—Cl^- ion exchange of chloragosomes. Chloragosomes were treated with 0.01 N-NaOH, centrifuged, washed 5 times with 5 ml water and subsequently with 1% $CaCl_2$. The pH of supernatants was measured. In other experiments 0.01% eosin solution was used instead of Cl^-.

Results

The trypaflavine-binding capacity of chloragosome fraction proved to be essentially higher than that of the body-wall homogenate (Fig. 1). The distribution quotients calculated on the basis of the data of Fig. 1, were $1-2.5 \times 10^1$ for the body-wall homogenate and 4.8×10^2 for the chloragosomes.

We assumed that in the binding mechanism of trypaflavine its cation character might play a decisive role and, on the other hand, that mechanism of binding is different in the body-wall homogenate and in the chloragosomes. For further investigations we started from the consideration that, depending on the binding forces, the rate of release of the bound trypaflavine as well as the quantities released into different media are different for the two kinds of tissues. The extracting media were added to the body-wall homogenates as well as to chloragosomes proportionally to the amount of bound trypaflavine. During aqueous extraction, a much smaller portion of the bound trypaflavine was released from the chloragosomes than from the body-wall homogenates (Fig. 2). Using Ca^{++}-containing washings on the other hand, almost all the

bound trypaflavine was released from body-wall homogenates into the first three washings, whereas from the chloragosomes a well measurable amount of trypaflavine was recovered even in the 18th washing fluid.

If 1% NaCl or 1% histidine solution was used as washing fluid, the trypaflavine release was less intensive than with $CaCl_2$. Furthermore, chloragosomes were found to bind atebrin and thionine essentially in a similar way as trypaflavine.

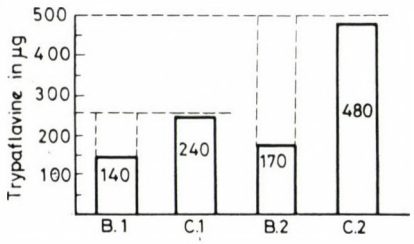

Fig. 1. Trypaflavine uptake by chloragosome fraction (C. 1 and C. 2) and by body-wall homogenate (B. 1 and B. 2) *in vitro*. B. 1 and C. 1 = uptake by 100 mg tissue from 250 µg trypaflavine, B. 2 and C. 2 = uptake by 100 mg tissue from 500 µg trypaflavine

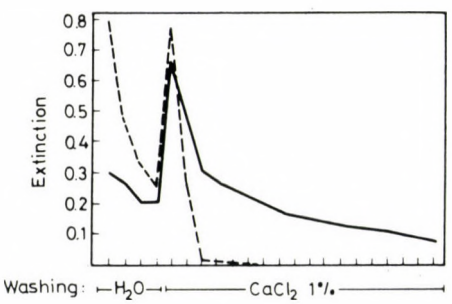

Fig. 2. Trypaflavine release by body-wall homogenate (— — —) and chloragosome fraction (————). Abscissa: consecutive washings. Ordinate: extinction of the washing fluids. The washings were carried out with 5 ml solutions at 5 min intervals

On the basis of the results it has been assumed that trypaflavine is bound to the body-wall homogenates mainly by VAN DER WAALS' forces, whereas the binding mechanism of chloragosomes is based on cation exchange.

The cation exchange character of chloragosomes has been proved by $H^+ - Ca^{++}$ ion exchange (Fig. 3). The chloragosome fraction transformed into H^+ form and subsequently washed acid-free, released H^+ ions on the effect of Ca^{++}, HCl was formed in the supernatant and the pH decreased. The H^+-liberation curve is of prolonged character resembling that of trypaflavine. If the trypaflavine-binding of chloragosomes is based on cation exchange, the chloragosomes transformed into H^+ form should also release H^+

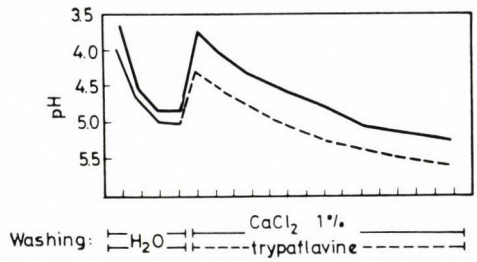

Fig. 3. The H^+ release of H^+-chloragosomes on the effects of Ca^{++} and trypaflavine. Abscissa: consecutive washings. Ordinate: pH of washing fluids

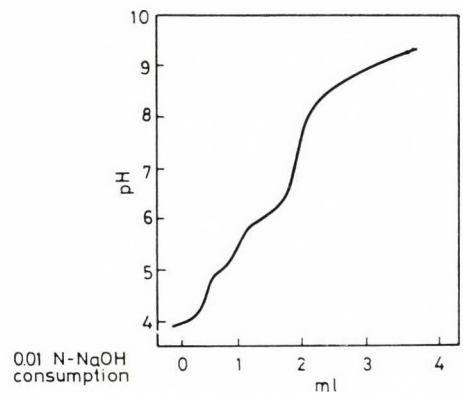

Fig. 4. The titrimetric curve of the chloragosome fraction

on the effect of trypaflavine. This supposition has been proved by the results of experiments (Fig. 3).

Conclusions can be drawn from the data of potentiometric titrations as regards the quality of active groups of the ion exchangers as well as the pH range where they are able to maintain ion exchange. Therefore, the titrimetric curve of the chloragosome fraction has been established (Fig. 4). This curve indicates that the chloragosomes are cation exchangers of polyfunctional character containing at least three different acid groups.

The question arose whether the chloragosomes had anion-binding or perhaps anion-exchanging properties. It was shown that the eosin-binding capacity of chloragosomes and body-wall homogenates was identical from a quantitative point of view. From the 500 μg eosin administered in 5 ml solution, 250 μg was bound by both tissues. The distribution quotient was 2×10 in both cases.

As to the release of the bound eosin, we have concluded that (Fig. 5)

(1) the chloragosome and body-wall homogenates essentially do not differ from each other from a point of view of extractions with distilled water;

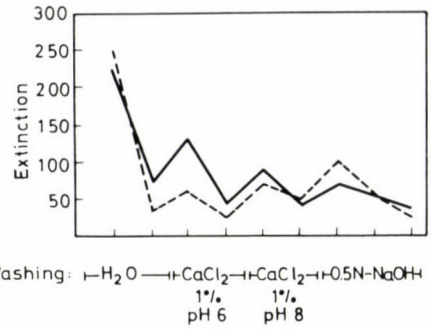

Fig. 5. The eosin release from body-wall homogenate (– – – –) and chloragosome fraction (————)

(2) the eosin release from the chloragosome fraction is significantly influenced by the ion concentration of the extracting medium, whereas that of the body-wall homogenate rather depends on the pH of the washing fluid.

The investigations concerning OH^- — Cl^- and OH^- — eosin ion exchanges gave a negative result in the chloragosome fraction. Therefore, the eosin-binding of chloragosomes is not based on ion exchange.

Discussion

Ion exchangers are insoluble in water and other simple solvents [9, 11]. According to several earlier reports [4—7, 16, 17, 20] and the present results, the chloragosomes fulfil this criterion. The basic property of ion exchangers is that they are polyelectrolytes containing active groups being able to dissociate [9, 11]. The cation exchangers are polyacids with carboxyl, phenolic hydroxyl, phosphoric or sulphonic acid groups, the polyfunctional cation exchangers possess several acid groups. On the basis of the titrimetric curve, the chloragosomes are polyfunctional cation exchangers displaying at least 3 kinds of acid groups. According to the investigations on the composition of chloragosomes [16, 17, 20] they contain phosphoric acid, carboxyl [4, 5], phenolic hydroxyl[6] and, probably in smaller amount, even sulphonic acid groups [7, 16]. The H^+ ions of cation exchangers can easily be changed for other cations. The possibility of exchange was proved in the present experiments in the chloragosome fraction, i.e., the cation exchanging role of chloragosomes, at least *in vitro*, can be accepted.

Even the anion-binding capacity of chloragosomes is considerable. According to our results, however, the anions are bound not with anion exchange but by adsorption to the substances of both chloragosomes and body-wall homogenate. The anions are probably linked to the acid groups of chloragosomes by adsorption and this can be the reason why, in the presence of $CaCl_2$,

they release the bound eosin more intensely than the body-wall homogenate does.

The occurrence of cation exchangers in living organisms was first reported by WHITE [22], demonstrating that role of melanin.

The physiological significance of the cation exchanging role of chloragosomes is indicated by the considerable mass and peculiar localization of the chloragogen tissue (Fig. 6). The chloragogen cells are situated along the dorsal

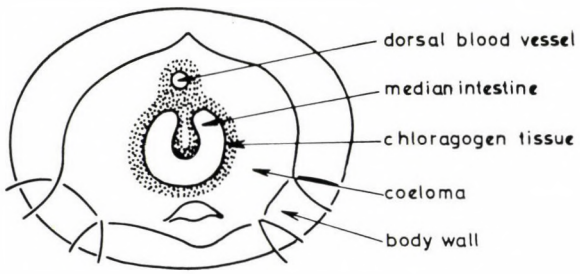

Fig. 6. The localization of the chloragogen tissue in the organism of Lumbricidae (transversal section)

blood vessel and on the surfaces of periintestinal blood sinuses [14], i.e., they separate from each other two spaces of fluids, viz., blood and coelomic fluid. These significantly differ from each other as regards ionic composition [13].

In our opinion the physiological effect of the ion-exchanging role of chloragosomes may play an important role in the following processes:

(1) the maintenance of a stable cation composition and pH in the fluids;

(2) the storage of organic cations, e.g., certain amino acids [5, 21];

(3) binding of toxic ions, i.e., protective effect as proved by us in *in vivo* experiments with trypaflavine and atebrin [7, 8]. According to WILLIAMS [23], the majority of toxic electrolytes are not detoxified in the animal organism but are directly excreted. In accordance with this, the ion exchangers in a living organism may keep toxic electrolytes at a low-level by binding them and releasing gradually.

(4) The cation exchangers may act as redox-catalysers by binding of metal ions of variable valencies [11]. The iron content of chloragosomes [7] as well as their participation in oxidative processes have been proved [8]. On this basis one can assume that their oxidative activity can be attributed to their iron content.

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Acta biol. Acad. Sci. hung., **24** (3—4), 165—170 (1973)

PECTINASE ACTIVITIES IN SOME FUNGI

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(Received 1973—03—16)

Abstract

Constitutive extracellular and intracellular pectinase activities by 10 fungal strains under different culturing conditions were investigated. The mycelial sporulation showed no correlation to pectinase activity. The sequence of extracellular enzyme activity differed according to the type of culture even for the same fungal strain. No correlation was found between the extent of intracellular pectinase activity and that of the culture filtrate of the same fungal strain. It is suggested that the extracellular and intracellular pectinase activities of the same fungal culture are different. An inverse relationship seems to exist between the yield of extracellular milk-clotting enzyme(s) and that of the pectinase enzymes for the investigated fungi.

Introduction

The importance of pectinases lies in their use in food and textile industries. Various microorganisms have been reported to produce these enzymes [7]. Preparations of extracellular and intracellular fungal pectinases have been reported [1, 4—6, 8].

ABDEL-FATTAH and co-workers [2] have recently reported on a distribution pattern of milk-clotting and proteolytic activities in some fungi. The present work was undertaken to investigate the pectinase activities in both extracellular and intracellular fungal enzyme preparations under different cultural conditions. Some emphasis was imposed on the correlation between milk-clotting and pectinase activities in fungi, a phenomenon which has not yet been reported.

Material and method

Microorganisms. The fungal strains used in the present work were obtained from NRRL except *Aspergillus niger* T, which is a local isolate taken from the collection of this Laboratory.

Culture medium. The medium used consisted of corn steep water (2% w/v) and lactose (2% w/v).

Cultivation. Transfers were made from the subcultures to Dox's agar plates which were then incubated at 30 °C for 7 days. Cultivation was done in 250 ml flat-bottomed flasks, containing 50 ml sterile medium. Four disks, 0.5 cm in diameter, were cut from the 7-day-old culture plates and used for inoculating flasks. After an incubation for different periods at 30 °C (surface and shaken cultures), the fermented medium was filtered and the culture filtrate of each flask was made up to 50 ml with distilled water. The pH value of the culture filtrate and the dry weight of mycelium were also determined.

Preparation of intracellular enzymes. This was done by grinding the air-dried and cooled mycelia with cold water (100 mg/g dry mycelia) for 10 min. The cell-free extract was then obtained by filtration through Whatman No. 50 paper.

Assay for pectinase activity. This was done by measuring the decrease in the viscosity of buffered citrus pectin solution. 0.4 ml of the enzyme solution was added to 9.6 ml of 0.5% citrus pectin solution (acetate buffer of pH 4.5) and the reaction mixture was incubated at 40 °C for 10 min. Thereafter, 5 ml of the reaction mixture were pipetted into an Ostwald viscosimeter in a water bath at 40 °C and the time of run was recorded. In control tests heated enzyme solutions were employed.

Protein determination. This was done according to the method of LOWRY and coworkers [9].

Results and discussion

Among the fungal cultures investigated, *Penicillium chrysogenum* had the least mycelial dry weight (Table 1). However, the culture filtrate of that organism contained the highest amounts of protein in both of its surface and shaken cultures as compared to culture filtrates of other organisms (Table 2). On the other hand, for either surface or shaken cultures no correlation existed between the mycelial growth and the protein content of the culture filtrate.

Table 1

The mycelial dry weight of fungal cultures

Time of incubation (days)	Surface culture			Shaken culture		
	3	7	10	3	7	10
Microorganisms	Dry weight of mycelium (mg/50 ml culture medium)					
Aspergillus flavus 482	402	627	955	284	635	726
Aspergillus niger T	980	616	554	779	786	749
Aspergillus niger 599	982	516	452	571	732	500
Byssochlamys fulva 2614	228	478	470	139	550	614
Chaetomium globosum 1870	156	380	487	321	415	547
Fusarium moniliforme 2284	280	478	550	234	523	523
Gliocladium roseum 1084	241	787	832	196	508	842
Mucor hiemalis 1419	148	317	386	411	515	483
Penicillium chrysogenum Wisco. *Q* — 176	19	58	81	40	321	453
Penicillium citrinum 805	465	575	441	257	587	527

As shown in Table 3, the extracellular pectinase activity of the different fungal cultures was influenced by the time of incubation as well as the type of culture. However, for any organism the pectinase activity during incubation did not depend on either the dry weight of the mycelium or the protein content of the culture filtrate. Furthermore, the pH of the culture filtrate was not characteristic of the pectinase activity. However, this sequence of extracellular

Table 2

The protein content of the culture filtrates of fungal cultures

Time of incubation (days)	Surface culture			Shaken culture		
	3	7	10	3	7	10
Microorganisms	Protein content (mg/ml)					
Aspergillus flavus 482	2.11	0.94	0.76	2.15	0.81	0.63
Aspergillus niger T	0.77	0.90	0.74	1.16	0.77	0.62
Aspergillus niger 599	0.86	0.87	0.81	1.63	0.60	0.57
Byssochlamys fulva 2614	3.22	1.57	1.21	3.05	1.15	1.17
Chaetomium globosum 1870	3.38	3.07	2.58	2.31	1.36	1.16
Fusarium moniliforme 2284	2.74	1.97	1.81	2.80	1.20	0.95
Gliocladium roseum 1084	3.15	1.15	0.92	2.90	1.62	1.07
Mucor hiemalis 1419	2.57	1.86	1.73	1.54	1.04	1.28
Penicillium chrysogenum Wisco. Q — 176	4.07	3.67	3.14	4.05	2.40	1.61
Penicillium citrinum 805	1.77	0.83	0.72	2.67	1.10	0.81

Table 3

The pectinase activity of the culture filtrates of fungal cultures

Time of incubation (days)	Surface culture			Shaken culture		
	3	7	10	3	7	10
Microorganisms	Pectinase activity (Reduction in viscosity) per cent					
Aspergillus flavus 482	9.89	13.52	2.11	14.37	7.25	2.63
Aspergillus niger T	52.09	44.54	40.37	50.36	49.70	48.18
Aspergillus niger 599	51.56	47.72	33.82	47.19	49.70	41.89
Byssochlamys fulva 2614	36.75	44.67	41.15	33.01	29.87	35.83
Chaetomium globosum 1870	0.33	4.99	9.78	6.20	11.41	13.10
Fusarium moniliforme 2284	7.62	7.06	7.32	8.65	12.38	16.07
Gliocladium roseum 1084	20.95	49.74	52.94	8.25	41.92	44.93
Mucor hiemalis 1419	40.44	51.78	55.86	48.97	54.38	20.23
Penicillium chrysogenum Wisco. Q — 176	7.49	17.05	17.05	11.74	25.70	39.67
Penicillium citrinum 805	2.83	0.26	1.64	5.01	1.18	0.00

pectinase activity differed according to the type of culture even with the same fungal strain. Although the extent of this constitutive extracellular enzyme activity was favoured with some fungal strains in surface cultures and with others in shaken cultures, yet the yield depended on the organism. Thus, while the culture filtrates of *Aspergillus flavus* and *Penicillium citrinum* showed weak pectinase activity, those of *Aspergillus niger* strains and *Penicillium chryso-*

genum were highly active. In addition to the latter fungal cultures, *Byssochlamys fulva*, *Gliocladium roseum* and *Mucor hiemalis* proved to be potent organisms for producing extracellular constitutive pectinase activity.

In a previous work [2], using the same fungal strains under the same cultural conditions, *Penicillium citrinum* proved to be a potent organism for producing active extracellular milk-clotting enzyme. Conversely, in the present work, *Penicillium citrinum* showed a weak, if any, extracellular pectinase activity. Further, either in surface or shaken cultures, *Penicillium chrysogenum* and *Gliocladium roseum* produced little or no extracellular milk-clotting enzyme [2], while the same culture filtrates of these organisms possessed high pectinase activity (Table 3). Generally, an inverse relationship seemed to exist between the yield of extracellular milk-clotting enzymes and that of pectinase enzymes. Furthermore, in contrast with the milk-clotting enzyme activity in several fungal species [3, 10], the pectinase activity was not associated with spore formation.

The cell-free extracts of most fungal cultures showed weak pectinase activity as compared to the culture filtrates (Table 4). Irrespective of the type of culture, no correlation existed between the extent of the intracellular pectinase activity and the mycelial dry weight. It was of interest, however, that the cell-free extracts of those organisms characterized by high extracellular pectinase activity showed the most intensive intracellular enzyme activity. This provided another evidence for the dependence of pectinase productivity on the fungal strain.

Table 4

The pectinase activity of the cell-free extracts of fungal cultures

Time of incubation (days)	Surface culture			Shaken culture		
	3	7	10	3	7	10
Microorganisms	Pectinase activity (Reduction in viscosity) per cent					
Aspergillus flavus 482	2.93	1.82	2.54	1.49	2.41	3.25
Aspergillus niger T	4.72	9.12	11.64	2.86	5.06	14.84
Aspergillus niger 599	1.99	19.36	19.63	2.46	15.37	25.35
Byssochlamys fulva 2614	7.07	10.71	22.60	8.46	9.06	7.07
Chaetomium globosum 1870	2.03	1.11	0.39	1.70	3.66	0.92
Fusarium moniliforme 2284	3.40	4.97	0.13	5.10	2.87	3.33
Gliocladium roseum 1084	0.59	10.42	6.71	2.24	4.56	7.23
Mucor hiemalis 1419	4.31	10.85	5.62	10.72	7.19	10.00
Penicillium chrysogenum Wisco. Q — 176	*	*	*	*	1.23	2.41
Penicillium citrinum 805	1.43	0.52	0.71	0.97	2.47	0.45

* The mycelial mats were not sufficient for investigation

As in the case of extracellular activity, the period of incubation as well as the type of culturing influenced the intracellular pectinase activity. However, it is worthy to note that no correlation was found between the extent of intracellular activity and that of the culture filtrate of the same fungal strain. Thus, in surface cultures of *Aspergillus niger* strains of the same age (3 days) the extracellular and intracellular pectinase activities showed their maxima and minima, respectively. Similar discrepancies were noted with other fungal cultures as well.

Table 5

The ratio of extracellular to intracellular pectinase activities of fungal cultures

Time of incubation (days)	Surface culture			Shaken culture		
	3	7	10	3	7	10
Microorganisms	*E/I***					
Aspergillus flavus 482	3.37	7.42	0.83	9.64	3.01	0.81
Aspergillus niger T	11.03	4.88	3.46	17.61	9.82	3.24
Aspergillus niger 599	25.91	2.46	1.72	19.20	3.23	1.65
Byssochlamys fulva 2614	5.20	4.17	1.82	3.90	3.29	5.06
Chaetomium globosum 1870	0.16	4.40	24.96	3.65	3.12	14.31
Fusarium moniliforme 2284	2.24	1.42	55.90	1.70	4.34	4.82
Gliocladium roseum 1084	35.51	4.77	7.87	3.68	9.19	6.21
Mucor hiemalis 1419	9.37	4.77	9.93	4.56	7.56	2.02
Penicillium chrysogenum Wisco. Q — 176	*	*	*	*	20.09	16.46
Penicillium citrinum 805	1.98	0.50	2.30	5.16	0.47	0.00

* The mycelial mats were not sufficient for investigation
** Extracellular/intracellular pectinase activity ratio

The data in Table 5 show that the ratio of extracellular to intracellular enzyme activities varied according to the organism and for the same fungal strain with the type of culture and even with the age of the same culture. This great variation may provide some evidence that the extracellular and the intracellular pectinase activities produced by each of the investigated fungi, in either surface or shaken culture, are different.

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Acta biol. Acad. Sci. hung., **24** (3–4), 171–174 (1973)

ISOLATION AND CHARACTERIZATION OF RIBOSOMES FROM STREPTOMYCES GRISEUS SPORES

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(Received 1973—04—12)

Abstract

Ribosomes from *Streptomyces griseus* spores were isolated and characterized by ultracentrifugal analysis. Sedimentation constants were calculated by comparison with parallel reference consisting of *Escherichia coli* ribosomes. The 70 S monomer dissociates into subunits with a sedimentation coefficient of 50 S and 30 S. Ribosomes derived from spores are more stable under the same ionic conditions (10^{-5} mol/l Mg^{2+} or 0.5 mol/l NH_4Cl) than ribosomes isolated from young vegetative hyphae. The purified spore ribosomes contain a dark-yellow pigment (or pigments). It was impossible to remove this material from the ribosomes by washing with high salt concentrations.

Introduction

The aim of these experiments was to isolate ribosomes from *S. griseus* spores and characterize their sedimentation behavior on sucrose density gradients.

WOESE, LANDRIDGE and MOROWITZ [12] found that *Bacillus subtilis* spores contained particles with sedimentation coefficients of 50 S and 68 S. They were not able to identify particles which had a 30 S sedimentation coefficient. BISHOP and DOI [2] isolated ribosomes from *B. subtilis* spores which had sedimentation properties and RNA composition similar to those of vegetative cell ribosomes. They demonstrated that the presence of an active ribonuclease and a protease in *B. subtilis* spores may account for the instability of ribosomes under ordinary isolation procedures.

In our experiments *S. griseus* spore ribosomes were stable during the isolation process and it was not necessary to add any nuclease or protease inhibitor.

Material and method

A streptomycin non-producing variant of *S. griseus* (No. 45-H) was used. A detailed specification of the strain was published elsewhere [10]. Spores were produced in filtered soybean medium, cultured at 27 °C for 3 days. Cultures were harvested, washed and suspended in distilled water. The suspension was sonicated for 10 min in ice bath with an MSE ultrasonic disintegrator (Measuring and Scientific Equipment Ltd, Power Unit No. 3 000) at an intensity of 1.4—1.5 A Ammeter position. Strain No. 45-H has a short life-cycle which terminates with abundant conidium production [11]. All vegetative forms were disrupted during the

ultrasonic treatment, whereas there were no morphological alterations in the spores [1]. After the ultrasonic treatment spores were washed three times with distilled water and stored at −20°C until used.

Preparation of ribosomes from spores. The washed spores were suspended in 0.01 mol/l Tris—HCl buffer (pH 7.5) containing 0.06 mol/l KCl, 0.01 mol/l magnesium acetate and 0.005 mol/l mercaptoethanol (buffer A), then ruptured by a Cell Homogeniser (MSK, B. Braun). Beads with a diameter of 0.1 mm were used for the break-down process. An operating time of 4 min at 4 000 rev/min was sufficient to break down 90—95% of the spores. The bottle containing the material was cooled in liquid carbon dioxide. It was possible to keep the temperature of the suspension below 5 °C even over extended shaking times. The efficiency of breaking was controlled by phase contrast microscopy.

After disruption, the homogenate was separated from the beads, treated with 5 μg/ml of DNase-I (Worthington Biochemical Corp.) for 15 min at 4 °C, and centrifuged twice at 20 000 *g*. Ribosomes were pelleted at 110 000 *g* for 3 h in an MSE Automatic Superspeed 50 T. C. centrifuge, then washed twice in 0.02 mol/l Tris-HCl (pH 7.5), 0.01 mol/l magnesium acetate, 1.0 mol/l NH_4Cl, 0.005 mol/l mercaptoethanol (buffer B).

The washed ribosomes were resuspended in buffers used for dialysis, clarified at 30 000 *g* for 15 min, then the concentration of ribosomes was adjusted to 100 A_{260} units/ml. 0.5-ml samples were dialyzed for 5 h against 0.5 l buffer A with 10^{-5} mol/l magnesium acetate, and 0.001 mol/l Tris (pH 7.5), with 0.5 mol/l NH_4Cl (buffer C), respectively. The outer solutions were refreshed at hourly intervals.

Sucrose-gradient-sedimentation analysis. 0.2 ml dialyzed samples were layered on 10-ml linear gradients of 5—20% sucrose (Serva Saccharose, free of Ribonuclease) in buffer as required. The centrifugation was carried out at 110 000 *g* for 3 h at 4°C in a 3 × 10 ml swing-out rotor. After centrifugation fractions of 20 drops were collected from the bottom of the tube by use of a piercing unit (MSE). The fractions were diluted to 3-ml with distilled water and measured at 260 nm in a Unicam SP 1800 Spectrophotometer (1 cm light-path).

Visible spectra of the ribosome preparations were recorded with the Unicam Spectrophotometer from 300 to 650 nm. The conditions for these assays are similar to those used by MIKULIK and co-workers [8].

Results and discussion

The sedimentation properties of *S. griseus* 45-H spore ribosomes, isolated and purified as indicated above are shown in Fig. 1 and Fig. 2. Partial dissociation into 50 S and 30 S ribosome subunits occurred when ribosome preparation was dialyzed against 10^{-5} mol/l Mg^{2+} for 5 h. Under the same circumstances ribosomes from young vegetative hyphae dissociated completely into 50 S and 30 S (unpublished data).

Dialysis of spore ribosomes against buffer C, for the same time period as before (5 h), caused almost complete dissociation of 70 S monomers into 50 and 30 S subunits (Fig. 2). In addition, while spore ribosomes retained their structural integrity during of the 0.5 mol/l NH_4Cl treatment, ribosomes from young vegetative mycelia became unstable under these circumstances and underwent some conformational alterations resulting in decreased sedimentation coefficients (not yet published). These results indicate that ribosomes from young vegetative mycelia treated with NH_4Cl behave more or less similar to those of *E. coli* ribosomes [3—6,9], while, as shown above, spore ribosomes remained intact during the same treatment.

The results of sedimentation profiles indicate that ribosomes from *S. griseus* 45-H spores in this respect are identical with those from vegetative cells and other *Streptomyces* species [4].

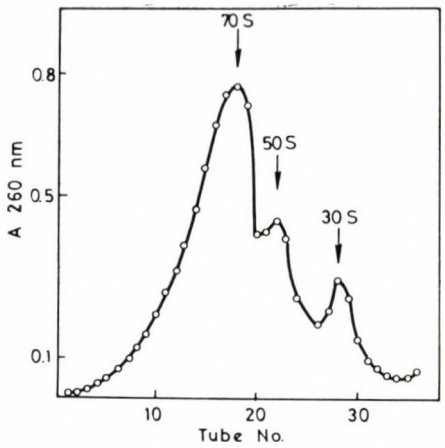

Fig. 1. Sucrose density gradient centrifugation of ribosomes from *Streptomyces griseus* spores. Ribosomes were isolated and purified, dialyzed for 5 h against buffer A with 10^{-5} mol/l Mg^{2+}, then centrifuged on a 5—20% sucrose gradient which contained the same buffer used for dialysis. Centrifugation was carried out at 110 000 *g* for 3 h. Fractions were collected, diluted and measured at 260 nm. Sedimentation is from right to left

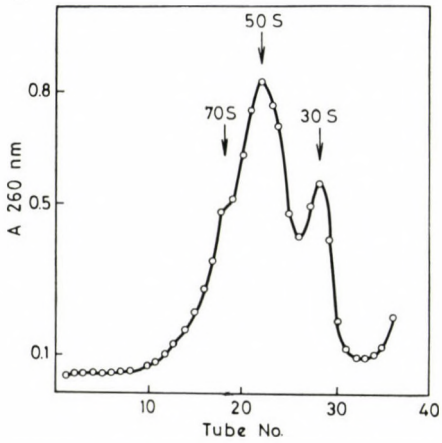

Fig. 2. Sedimentation pattern of NH_4Cl-treated spore ribosomes. Ribosomes were isolated and purified, dialyzed for 5 h against buffer C (0.5 mol/l NH_4Cl in 0.001 mol/l Tris, pH 7.5) then layered on a 5—20% sucrose gradient prepared in buffer C, centrifuged at 110 000 *g* for 3 h. Sedimentation is from right to left

S. griseus 45-H spore ribosomes contain some dark-yellow pigment(s), which is almost completely absent from the young vegetative hyphae. Fig. 3 shows the visible spectra of ribosomes isolated from spores and vegetative mycelia. The pigment(s) has an absorption maximum at 414 nm, and is insoluble in 0.5 or 1.0 mol/l NH_4Cl buffers.

The characteristics and the role of this pigment played in the stability of ribosomes of this streptomycin-non-producing strain is being investigated.

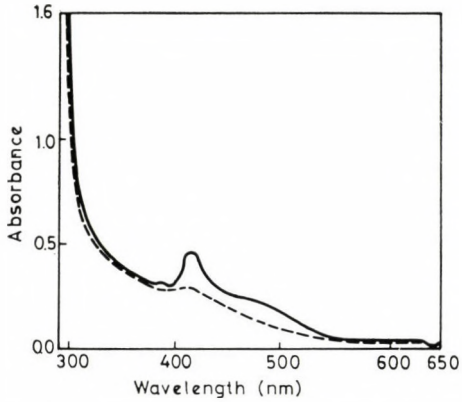

Fig. 3. Visible spectra of ribosomes isolated from spores and 16-h mycelia of *Streptomyces griseus* 45-H as indicated in the text. – – – – –: ribosomes from mycelia; ————: ribosomes from spores

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Acta biol. Acad. Sci. hung., **24** (3—4), 175—189 (1973)

ULTRASTRUCTURE, PIGMENT CONTENT AND PHOTOSYNTHETIC ACTIVITY OF THE NORMAL AND MUTANT CHLOROPLASTS IN DEVELOPING TRADESCANTIA LEAVES

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(Received 1973—04—26)

Abstract

In the course of ageing, in normal chloroplasts of the leaves of *Tradescantia albiflora* cv. *aureo-vittata* (from the 1st—5th to the 10th—15th leaf, as numbered from the shoot apex) the lamellar system enriches and the pigment content increases. In contrast with this, the CO_2 fixation decreases, which is brought by the authors into connection with a functional rearrangement of the photosynthetic units and with the reduction of the stroma substance. The young mutant plastids have variable structure containing grana-like bodies, single lamellae and vesicles of different size. During the ageing, their inner membrane system becomes characteristically destructed, which is followed by a rapid decrease in the chlorophyll content and a slower decrease in the CO_2 fixation. In the meantime, photosynthetic units decrease in both size and number. Two kinds of compensating mechanisms seem to be activated in the mutant: (1) the pigment system becomes more efficient and (2) the dark fixation of the CO_2 increases.

Introduction

Within the young leaves of *Tradescantia albiflora* cv. *aureo-vittata* there alternate green and yellowish-greenish streaks, the mesophyll of which contains normal and defective chloroplasts, respectively.

Whilst the normal chloroplasts of the mesophyll are light-microscopically uniform (except for their moderate variability in size), the total of the mutant plastids is conspicuously heterogeneous with regard to size, shape, inner structure as well as pigment content [9]. On the basis of our light-microscopic observations [9] we have characterized the outstanding types of the mutant plastid set and have stated that their occurrence rate changes during the ageing of the leaves. Namely, there is a gradual decrease in number of the plastids containing discoidal bodies rich in pigment, whilst the colourless types of a granular-foamy structure show an increasing frequency. This process becomes visible to the naked eye by the whitening of the pale streaks that proceeds sooner by strong illumination and slower in shadow.

The alteration in type rate was obviously attributable to the destruction of the green mutant plastids, however, the developmental relations of the

different types, namely, the primary or secondary character of the heterogeneity as well as the ways of destruction, were detectable only by electron microscopy. Considering the contradictions as regards the localization of the photosynthetically active pigments within the chloroplast, it seemed to be justified to examine the ultrastructure of the mutant pigment-bearing structures in connection with the measurement of the pigment content and pigment composition, as well as with that of the photosynthetic activity in cases of the different type rates. Last but not least, we wanted to study the interrelations between structure and function also in the normal chloroplasts of the leaf in the developmental stage corresponding to the destruction of the mutant plastids.

Material and method

Material and sampling: The experiments were carried out on the leaves of *Tradescantia albiflora* KUNTH em. BRÜCKN. cv. *aureo-vittata* [4]. The plants were grown in flowerpots in the glass-house of the Biological Station of Eötvös Loránd University in Göd. The sampling was made according to morphological observation; from each sprout those leaves were included into the young normal and the young mutant group in which the pale streaks were relatively most green; the aged normal and the aged mutant group contained the leaves in which the pale streaks had already been whitened, but the green streaks showed no visible change of colour. According to these criteria, the 1st to 5th leaves, as numbered from the shoot apex, were generally qualified as "young" and, approximately, the 10th to 15th as "aged".

Electron microscopy: The leaf primordia (1—3 mm in size) were prepared from uniformly normal and mutant sprout tips under stereomicroscope. The material was infiltrated in a 1-per cent buffered $KMnO_4$ solution (pH = 7.4) and then fixed in it for 2 hours at room temperature. The fully developed young and aged striated leaves were infiltrated in tap-water and then their pieces, 1 mm in width, were fixed similarly to the leaf primordia. Fixation began between 9 and 10 a. m. in all cases. The materials were dehydrated in ethanol and embedded in Durcupan-ACM (Fluka). The sections made with the Porter-Blum MT—1 ultramicrotome were stained with lead citrate [11] and examined in the KEM 1 electron microscope.

Pigment analysis: From the green and pale streaks of the young and aged leaf-blades the pigments were extracted with acetone and then measured in ethylether using the Unicam SP 500 spectrophotometer. Chlorophyll a and b beside each other were determined by the two-wavelength method [5]. The calculations were made on the basis of the following molar extinction coefficients (l/mol × cm):

chlorophyll a: $D_{663\ nm} = 88\ 633$ $D_{644\ nm} = 14\ 117$
chlorophyll b: $D_{663\ nm} = 4\ 748$ $D_{644\ nm} = 56\ 265$

In the same solution the carotenoids were measured as β-carotene at 480 nm. The extinction coefficient of the β-carotene determined in ether at 480 nm was calculated from the extinction coefficient of the hexanic solution ($E^{1\%}_{1cm} = 2\ 580$) given by ZSCHEILE [22] and from the extinction rate of the pigment of equal quantity determined in the two solvents at the given wavelength. In the result, the coefficient in question was found to be $E^{1\%}_{1cm} = 1\ 980$.

The pigment content was calculated not only for the weight unit of the fresh leaf material but also for identical cell number, because the weight unit of the aged leaves contains much fewer cells — as a consequence of the cell growth — than that of the young leaves. As cell number unit the average number of the chlorenchyma cells (K) was chosen which was contained in 1 g of the fresh weight of the young normal leaves. According to our measurements, in the aged leaf the chlorenchyma cells of number K are contained in 2.1 g fresh weight.

The carotenoid composition was examined by thinlayer chromatography. The layers and the solvent-systems were made according to HAGER, MEYER-BERTENRATH [8]. The relative intensity of the stripes was determined by densitography of the photographs of the chromatograms.

Measurement of the photosynthetic activity: The photosynthetic activity was characterized by the CO_2 quantity incorporated by 1 g leaf material (dry weight), and by cells of number K, respectively, within two hours at an illumination of 5 000 lux. The leaf-blades laid on wet filter paper (with their adaxial surface upwards) were placed into an illuminated closed system [7], in which the CO_2 concentration was 0.06 per cent, the ^{14}C activity 235 μCi/2 l. After exposure, the leaves were cut to normal and mutant strips, then discs of equal size were cut out of them. The incorporated CO_2 per disc was calculated by measuring the radioactivity of dried discs of known weight (8 to 12 discs per group), taking into consideration the specific activity of the CO_2 (4.80 μCi/μmol).

Results

Normal chloroplasts

Ultrastructure: In the examined youngest leaf primordia (3 mm in length) the plastids already contain expanded lamellae gathered into several bundles (Fig. 1). A single bundle is made up of 6—8 lamellae maximally; thickness of the lamellae reaches that of the developed young plastids' grana-lamellae (see Fig. 4). It is important, as regards our further examinations, that the plastid set is homogeneous concerning the degree of differentiation. Only few dividing plastids were found, all in dividing cells (Fig. 2).

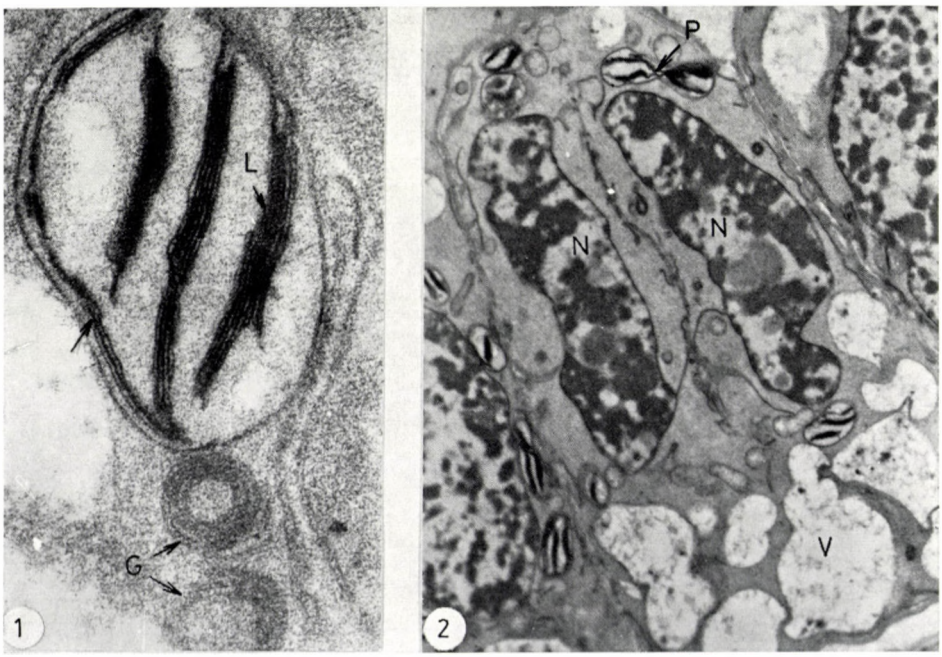


Fig. 1. Plastid in a leaf primordium 3 mm in size. *L* = lamellar bundle; ↓ = dichotomy of a lamella; *G* = Golgi apparatus; × 30 800

Fig. 2. Cell division and plastid division in a normal leaf primordium 3 mm in size. *P* = dividing plastid; *N* = nucleus; *V* = vacuole; × 4 400

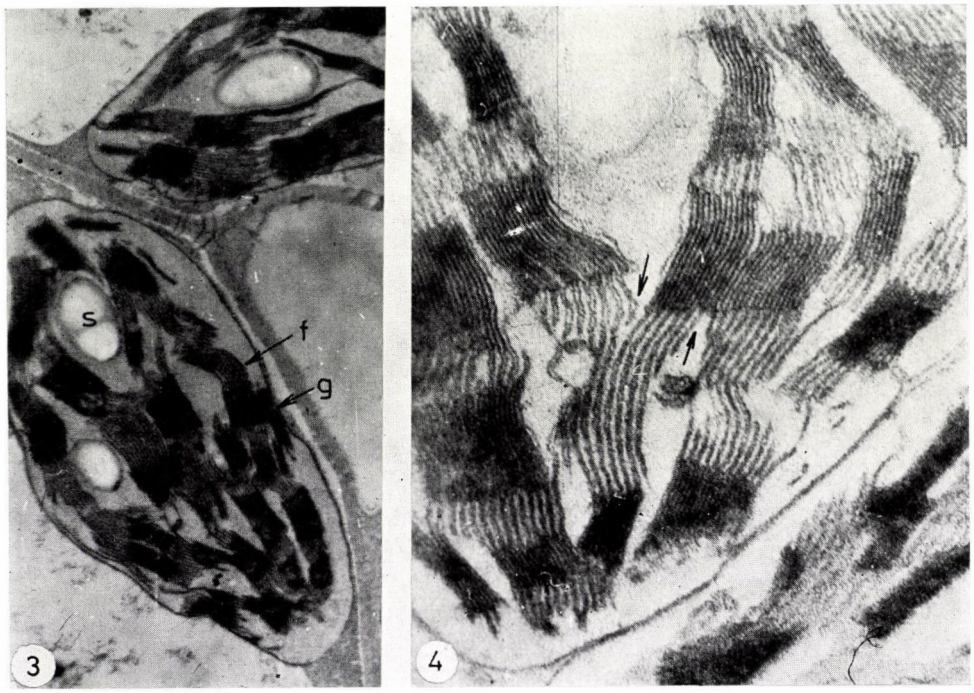


Fig. 3. Chloroplasts within the normal (green) streak of a young leaf. g = granum; f = fret membranes; s = starch; × 13 200

Fig. 4. Part of a young normal chloroplast. ↓ = lamellar bundles possibly in the stage of junction; × 30 800

In young leaf samples the inner membranes of the plastids still show discernible bundled arrangement (Figs 3, 4). In section, each bundle contains 2—3 grana that are built up of 10—25 thylakoids. The grana are horizontally connected with each other by fret membranes travelling regularly; the number of these is half as much as that of the thylakoids within the joining grana. The bundles are loosely connected with each other, leaving a wide space for the stroma substance.

Within plastids of the aged leaf group the lamellar system becomes more rich and, as a consequence of this, the stroma substance reduces strongly (Fig. 5). The number of thylakoids per granum may exceed 30, which, in certain cases, can be attributed to the fusion of adjacent bundles. Thickness and contrast of the membranes generally increase in the grana and the fret (Figs 4, 5). In some cases, however, local disorganization of thylakoids was observed (Fig. 6).

Pigment content: In the course of ageing both the chlorophyll and the carotenoid level decreases as calculated for the weight-unit of the leaf material, but increases as calculated for equal cell number or — which means the same in this case — for equal plastid number (Table 1). Although there is no doubt

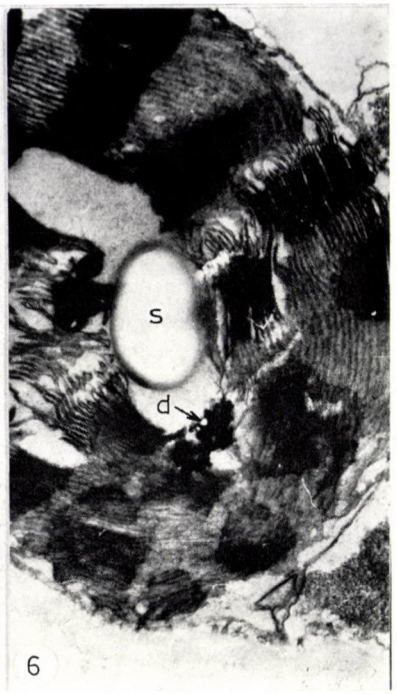


Fig. 5. Part of an aged normal chloroplast. t = a granum-thylakoid seen from its face; m = a granum sectioned through its margin; × 30 800

Fig. 6. Aged normal chloroplast. d = disorganization of membranes; s = starch; × 22 000

that the latter reference is the right one and the former may lead to considerable errors, we have given the data also for the weight unit as usual in the literature.

In the phase of plastid development examined by us the whole pigment level increases by about a half of its value, whilst the rates of the different pigments show a little change. The quantity of the xanthophylls grows most intensely; the two chlorophylls show a lesser but parallel increase, whilst that of the carotenes is the smallest.

Photosynthetic activity: In the course of ageing the CO_2 fixation decreases significantly, to about its half, even if calculated for equal cell number. Dark fixation is negligible (Table 2).

Efficiency of the pigment system: The size as well as the number of the photosynthetic units can be calculated from the chlorophyll content and the CO_2 fixation [21]. As demonstrated in Table 3, the size of the unit (that is, the number of the chlorophyll molecules required for the transport of a single electron) increases during the ageing, which means a decreasing efficiency. Numerically, this can so be interpreted that the size of the young unit (290)

Table 1

Pigment content of normal leaf segments

	In young leaves	In aged leaves	
	per 1 g fresh weight = per cells of number K	per 1 g fresh weight	per cells of number K
Chlorophyll a nmol	906.4	679.7	1 427
Chlorophyll b nmol	517.0	385.2	809
Total chlorophyll nmol	1 423.4	1 064.9	2 236
per cent	100	74.8	157
Chlorophyll a / Chlorophyll b	1.8	1.8	1.8
Total carotenoid nmol	305.6	244.4	513
per cent	100	79.9	168
Carotene / Xanthophyll	0.70	0.55	0.55
Chlorophyll / Carotenoid	4.7	4.3	4.3

Table 2

CO_2 *fixation by normal leaf segments*
(L = in light, D = in darkness. In all cases $P < 0.02$)

			In young leaves	In aged leaves	
			per 1 g dry weight = = per about 10 K cells	per 1 g dry weight	per about 10 K cells
Incorporated CO_2	L	μmol	4 435 $\pm$ 1 409	1 126 $\pm$ 371	2 365 $\pm$ 779
		per cent	100	25.4	53
$\bar{x} \pm S\bar{x}$	D	μmol	24.5 $\pm$ 6.6	8.1 $\pm$ 1.0	17 $\pm$ 2.1
		per cent	0.5	0.2	0.4

increases to about 440 as a consequence of the pigment enrichment (see Table 1) and, simultaneously, every second unit becomes inactive, however, their pigment content is comprised in the aged units (863 chlorophyll molecules). Accordingly, the number of the units decreases to about its half, as calculated for the same cell number.

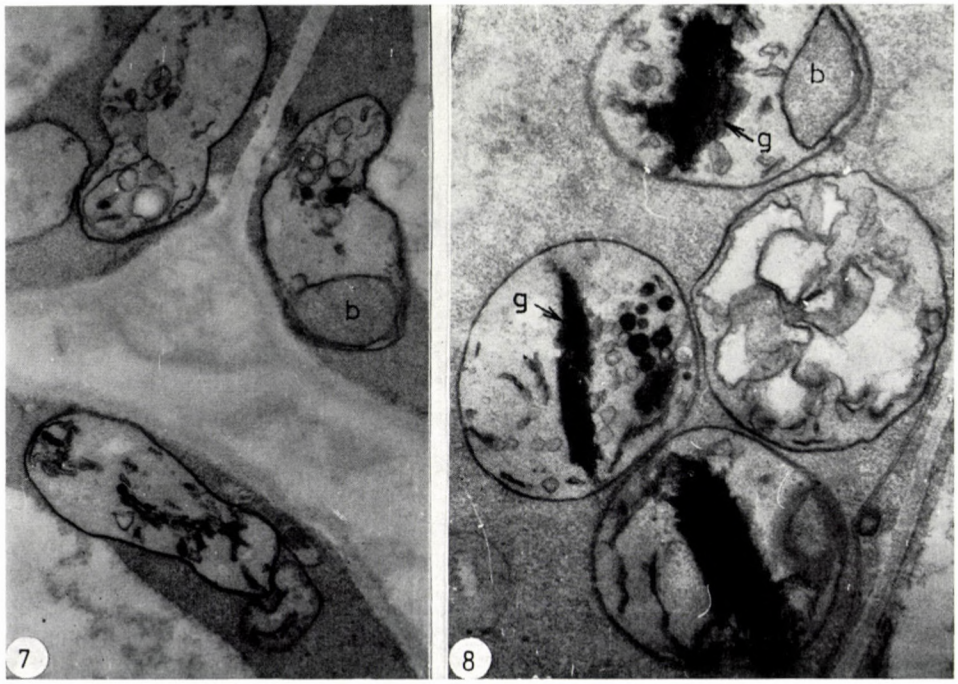
Table 3

Photosynthetic units of normal plastids

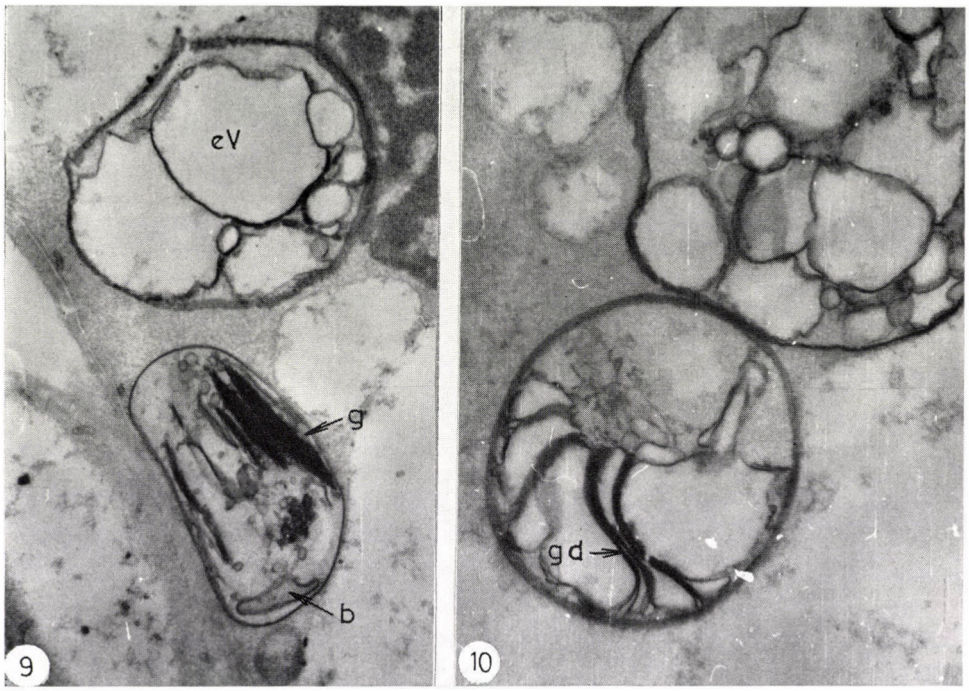
		In young leaves	In aged leaves
Size		290	863
Number	per 1 g dry weight	306×10^{14}	77×10^{14}
	per about 10 K cells		162×10^{14}

Mutant chloroplasts

Ultrastructure: In the mutant leaf primordia, 1 mm in size, the plastid set is already heterogeneous. Some plastids contain small vesicles, dense droplets, single thylakoids and bodies of cytoplasmic density limited by a membrane (Fig. 7); other plastids have, in addition, very dense, condensedly membranous structures (Fig. 8). In leaf primordia, 3 mm in size, also large plastids can often be found which contain dilated vesicles (Figs 9, 10). These vesicles are formed by a disintegration of dense membrane bundles (Fig. 10). Thus the plastid set becomes heterogeneous by a secondary way, too.



Figs 7 and 8. Plastids in mutant leaf primordia 1 mm in size. *b* = body of cytoplasmic density; *g* = dense membrane bundle; × 22 000



Figs 9 and 10. Plastids in mutant leaf primordia 3 mm in size. *ev* = expanded vesicle; *gd* = dilating dense membrane-bundle; for other marks see Fig. 8; × 13 200

The mutant plastid set of the young leaves includes various structure types (Fig. 11). Plastids containing one flat body or more and single lamellae are still frequent. The dense body seems to consist of narrow thylakoids (Fig. 12), so it may be qualified as the mutant homologue of the normal granum. In cases of certain plastids it is difficult to decide whether their lamellae adjoining along shorter or longer part originate from a destructed bundle, or they never had formed granum-like body (Fig. 13). In the case of the type demonstrated in Fig. 14, the destruction is probable, seeing that some of its lamellae are fragmented into vesicles. The more frequent way of the destruction of granum-like bodies is a dilatation in the course of which the inner space of certain thylakoids enlarges without a simultaneous ceasing of the connection between the thylakoids (Figs 15, 16). Such plastids contain as a rule numerous electron transparent vesicles, which may have been formed by fragmentation of fret-like membranes (see Fig. 12).

In mutant plastids of the aged leaf no granum-like bodies are found and also the frequency of the lamellae decreases to a minimum. Certain plastids are rich in small electron dense vesicles, which can be regarded as fragments of structures abounding in lipids (Fig. 17). In most plastids, however, large

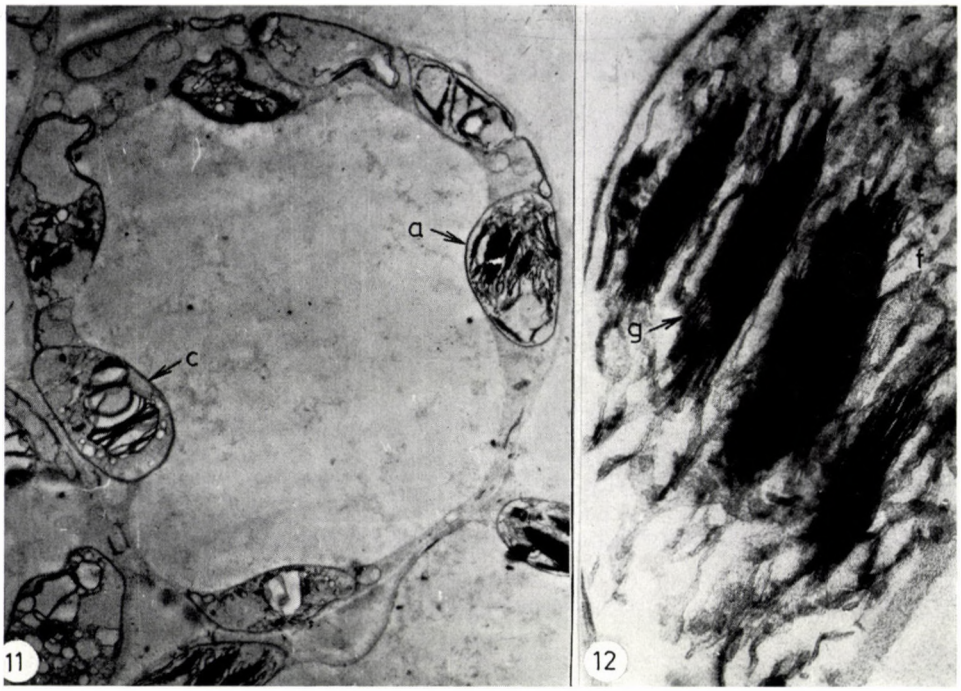


Fig. 11. Heterogeneous plastid set in the mutant streak of a young leaf. *a* = a plastid with granum-like bodies; *c* = plastid under destruction; × 4 400

Fig. 12. Plastid with granum-like body (*g*) and fret-like membranes (*f*); × 31 400

transparent vesicles are dominating, until finally of the membranes only the peristromium remains visible (Fig. 18).

Pigment content: The pigment content of the mutant leaf segments, being small from the beginning as compared to the normal ones, decreases significantly in the course of ageing (Table 4). The chlorophyll/carotenoid ratio is low as early as the young developmental age, and mainly the chlorophylls are affected by the subsequent destruction as well. In the thin-layer chromatograms of the carotenoids no qualitative difference could be observed in comparison with the normal material. It should be noted that the true pigment content of the mutant plastid set must be somewhat below the measured one because the leaf segments contain also the normal chloroplasts of the stomata [comp. 16].

Photosynthetic activity: The CO_2 fixation is low in the mutant leaf segments already in the young developmental age (Table 5), though not as low as the chlorophyll level (comp. Table 4), and it decreases moderately as calculated for constant cell number during ageing. At the same time, dark fixation increases more than tenfold, that is, to a much higher level than can be

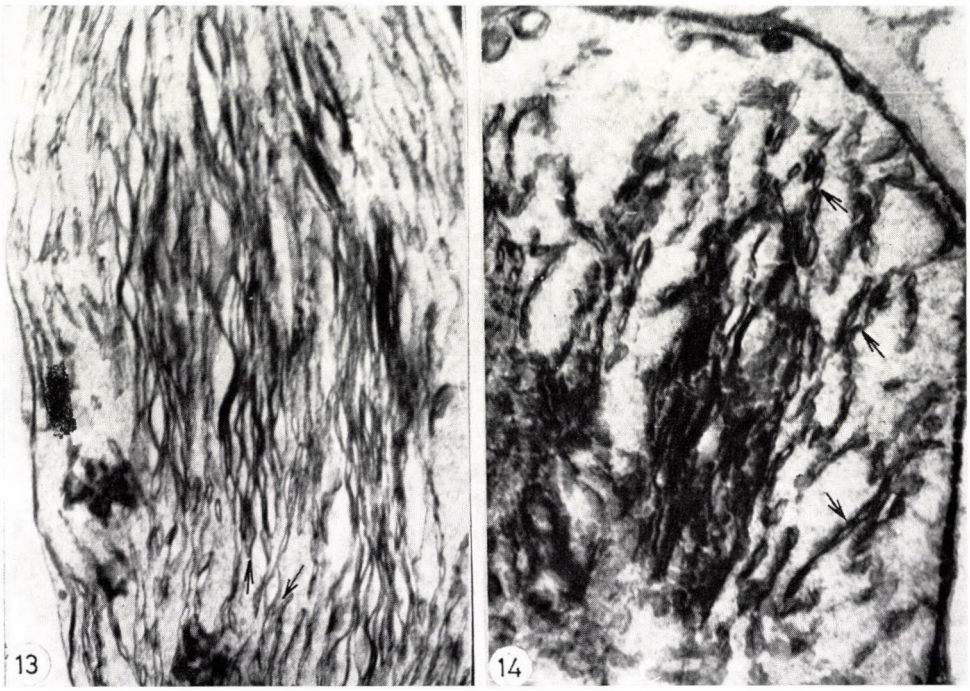


Fig. 13. Plastid with lamellae adjoining at some places (↓); × 44 000
Fig. 14. Plastid with fragmenting lamellae (↓); × 30 800

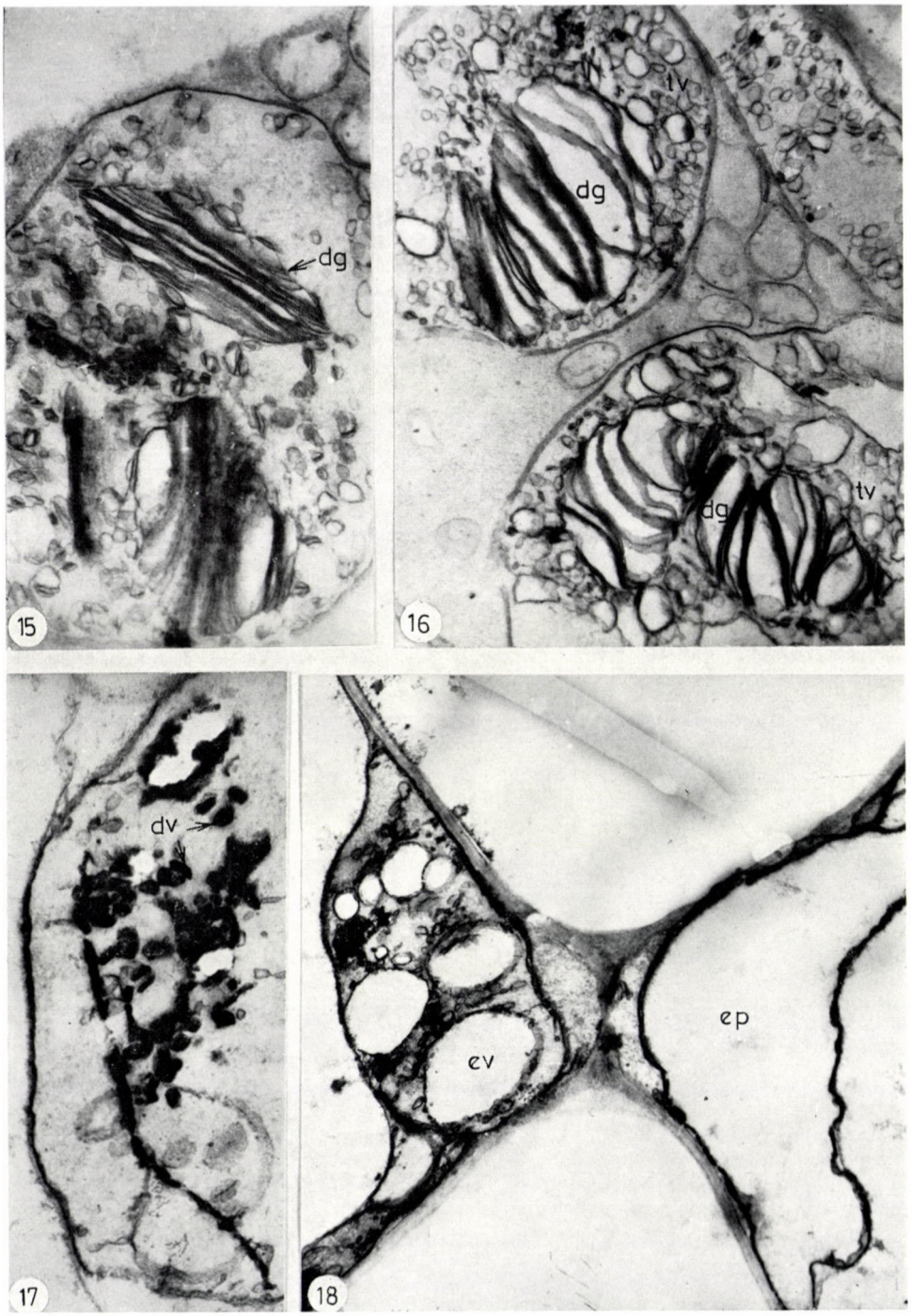
measured in normal leaf segments (comp. Table 2). In evaluating the data the presence of the guard-cells must be considered here too (see above).

Efficiency of the pigment system: According to Table 6, in the young mutant plastid set the transport of a single electron needs about half as many chlorophyll molecules as in the normal one (comp. Table 3), and this number decreases further in the course of ageing. This relatively great efficiency cannot be realized in absolute sense, because of the very low number of the units.

Discussion

Normal plastids

In the normal plastids the intense decrease of the CO_2 fixation, in the course of ageing, seems to be in contradiction with the increase in the pigment content and the enrichment of the lamellar system. We have seen, however, that in the mutant leaf segments units of about 60 chlorophyll molecules are functioning; this points to that the normal plastids have a very large pigment reserve, which gradually grows in time. As for the functional rearrangement of



Figs 15 and 16. Plastids containing dilating granum-like bodies (*dg*). *tv* = transparent vesicles; × 22 000 and 13 000

Fig. 17. Plastid containing electron dense vesicles (*dv*) in mutant streak of an aged leaf; × 22 000 *Fig. 18.* Plastid with expanded vesicles (*ev*) and an "empty" plastid (*ep*); × 13 200

Table 4

Pigment content of mutant leaf segments
(in brackets, per cent values in relation to the young normal material)

		In young leaves	In aged leaves	
		per 1 g fresh weight = per cells of number K	per 1 g fresh weight	per cells of number K
Chlorophyll a nmol		54.8	7.8	16
Chlorophyll b nmol		32.8	4.0	8
Total chlorophyll	nmol	87.6	11.8	24
	per cent	100 (6.2)	12.9 (0.8)	27 (1.7)
Chlorophyll a / Chlorophyll b		1.7	1.9	1.9
Total carotenoid	nmol	35.3	10.1	21
	per cent	100 (11.5)	28.7 (3.3)	60 (7)
Carotene / Xanthophyll		0.35	0.31	0.31
Chlorophyll / Carotenoid		2.5	1.2	1.2

Table 5

CO_2 *fixation by mutant leaf segments*
(L = in light; D = in darkness. In all cases, $P < 0.05$. In brackets, per cent values in relation to the young normal material)

			In young leaves	In aged leaves	
			per 1 g dry weight = = per about 10 K cells	per 1 g dry weight	per about 10 K cells
Incorporated CO_2	L	μmol	597 $\pm$ 244	197 $\pm$ 57	414 $\pm$ 120
		per cent	100 (13.5)	33.0 (4.4)	69 (9)
	D	μmol	5.3 $\pm$ 1.2	25.9 $\pm$ 3.5	54 $\pm$ 7
$\bar{x} \pm s_{\bar{x}}$		per cent	0.9 (0.1)	4.3 (0.6)	9 (1.3)

Table 6

Photosynthetic units of mutant plastids

		In young leaves	In aged leaves
Size		133	62
Number	per 1 g dry weight	41×10^{14}	12×10^{14}
	per about 10 K cells		25×10^{14}

the many small units into fewer and larger ones, this is possibly connected with a disturbed energy transport between the pigments. Enrichment of the lamellar system is in accordance with the increase of the pigment content and points to the reduction of the stroma substance. This, evidently, restricts the functioning of the carbon cycle and thus may, in itself, reduce the CO_2 fixation.

Mutant plastids

On the basis of the theory of the monotropic plastid development, several authors take the pale plastids of the panachure leaves for leucoplasts [6], whilst others mention them under the name of "albicate Plastiden" [15]. In our opinion, the term *mutant chloroplast* can be regarded as the right one, considering that a part of the plastid set is green and does photosynthesize for a while and, further on, that the plastids, when being destructed, do not change their function parallel with their decreasing photosynthesis.

As early as their starting developmental phase, the plastids show structural abnormalities, in contrast with the *Oenothera*, *Nicotiana* and *Humulus* mutants studied by v. WETTSTEIN [20]. In our plastids, from among the most conspicuous abnormal structures the bodies of cytoplasmic density covered by a limiting membrane as well as the primary electron dense droplets are characteristic only of the early phase of the development (leaf primordium of 1—3 mm in size). They presumedly contain those proteins and lipids which could not be organized into membranes continuously. The dense bodies of packed lamellae exist for a longer period of the development. They can be regarded as homologues of the normal grana on the following basis; 1. they are built up of thylakoids attached to each other; 2. they must comprise most part of the pigments, for they are electron dense and their destruction leads to a considerable decrease in the pigment level. Similar structures have been described by several authors in the plastids of various mutant plants, or in plants having disharmonious genome and plastome (e.g., DÖBEL in the status *albomaculatus* of *Antirrhinum majus* [3], RÖBBELEN in the xantha-mutant of

Arabidopsis thaliana [13], BACHMANN, ROBERTSON and BOWEN in the pastel 8 686 mutant of *Zea mays* at a temperature of 27—35 °C [2], SCHÖTZ and DIERS in *Oenothera* hybrid [14]).

Since the destruction of the plastids affects simultaneuosly both the structure and the pigment content, it is difficult to tell which of the membrane components represents the point of attack. According to our earlier observations, the whitening of the mutant segments is accelerated by intense illumination, however, this effect cannot be explained by the lack of light-protecting carotenoids [see 1, 17, 18], because in our mutant plastids more carotenoid molecules fall, on the avarage, to one chlorophyll molecule than in the normal ones. Also the dilatation way of the destruction of the granum-like bodies points to that the destruction within the thylakoids precedes that in partitions [19]. It is remarkable that these dilating plastids are morphologically quite similar to the *Funaria* chloroplasts treated with chloramphenicol by MLODZIANOWSKI, SZWEYKOWSKA and SCHNEIDER [10].

The heterogeneity of the plastid set is primary, which is in accordance with our statement that the mutation affects the plastome [9]. The primary heterogeneity points to that the mutation may be of different degree and thus supports the possibility of the polygeny or multiplex allelism regarding the affected plastid features within the plastome [comp. 12].

The comparison of the structure, pigment content and photosynthetic activity of the plastid sets of different age shows that the active pigments are located also in the mutant chiefly in partitions and in the course of the ageing the decrease in the functioning is caused (in contrast with the normal plastids) by the destruction of the photosynthesizing membranes. It seems that the mutant "tends to compensate" its defect in two ways: it reduces the size of its photosynthetic units and, in older age, it strongly increases the dark fixation of CO_2.

Acknowledgement

Authors express their thanks to Dr. L. FRIDVALSZKY (head of the Electron Microscope Laboratory of the Institute for Applied Botany and Histogenetics) for his precious help, to Dr. G. HORVÁTH for the thin-layer chromatography of carotenoids, to Mrs. P. PETROVITS for her valuable microtechnical work and to Mr. P. HANCKE for cultivation of the plant material.

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Acta biol. Acad. Sci. hung., **24** (3—4), 191—199 (1973)

CHARACTERISTICS OF CATECHOLAMINE FLUOROPHORES IN THE GANGLIA OF THE BIVALVE ANODONTA CYGNEA L. AS REVEALED BY A SIMPLE METHOD OF MICROSPECTROFLUOROMETRY

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(Received 1973—05—25)

Abstract

The fluorescence induced by the Falck—Hillarp technique was investigated in the neuropile of cerebral and pedal ganglia of *Anodonta cygnea*. It has been established that the green or greenish-yellow fluorescence of the axons originates exclusively in catecholamines and even among them dopamine predominates. A simple method of microspectrofluorometry was developed, using a cytophotometer type Leitz MPV. Continuous interference filters were applied in order to achieve monochromatic lights during the registration of excitation and emission spectra. The necessary correction factors have also been determined.

Introduction

The fluorescence-microscopic method of Falck and Hillarp [8] based on formaldehyde vapour treatment brings useful information even during the usual subjective microscopic observation as regards the distribution of the monoamines. However, the subjective observation does not enable us to decide with certainty which monoamine, or perhaps mixture of several monoamines, gives the origin of a certain fluorophore. There occur relatively large quantities of catecholamines (CA) and serotonin (5HT) in the ganglia of molluscs [5, 6, 7, 11, 12, 18, 23, 25, 26, 27, 28]. The localization of 5HT has not been cleared up, even at the cellular level. It has been supposed that the 5HT might be present in the neuropile together with CAs, therefore, the yellowish-green or green fluorescence of the neuropile originates in the mixture of fluorophores or both of them.

Such problems can only be solved by means of microspectrofluorometry enabling us to conclude to the origin of fluorophores on the basis of the excitation and emission spectra. The development of that method has taken place during the recent years and now several very precise instruments are used for this purpose [1, 3, 15, 19, 22].

However, those methods of microspectrofluorometry, being rather expensive, are not accessible to every laboratory. Therefore, we attempted to

find simpler, yet reliable methods [24]. The method described in this paper has proved to be suitable to answer the question whether the CAs and 5HT take part together in the formation of fluorophores in the neuropile of the ganglia in *Anodonta cygnea*.

Material and method

Adult bivalves of 14—18 cm body length were used. The cerebral and pedal ganglia were prepared according to FALCK and OWMAN [9] with some modifications [26]. Sections of 10 μm thickness were cut from the freeze-dried, formaldehyde-treated and paraffin-embedded ganglia, stretched out on warm, usual glass slides and covered with paraffin oil. The visceral ganglion was omitted, since its 5HT content proved to be the lowest among the ganglia [11].

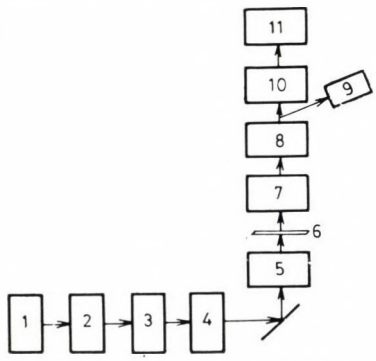

Fig. 1. Schematic diagram of the instrument for recording of excitation spectra. 1 = DC-operated XBO 150 xenon lamp; 2 = diaphragm; 3 = primary filters: BG 38/4 (Leitz) and BG 3/4 (Zeiss, Jena); 4 = continuous interference filter (CIF) (Schott, Mainz); 5 = glass condenser, n. a.: 0.65; 6 = specimen; 7 = objective lens, Pl. × 25, n. a.: 0.50; 8 = barrier filter K 510 (Schott, Mainz); 9 = prism for the binocular tube; 10 = projective lens, × 10; 11 = photomultiplier tube RCA 1P—21 with light diaphragm

The microspectrofluorometric measurement was carried out using dia-illumination by means of a Leitz MPV cytophotometer applied to a Leitz Ortholux II microscope. The photomultiplier tube was of RCA 1P—21 type. The relative light intensities were recorded from a galvanometer.

Recording of excitation spectra. The diagram of the instrument is shown in Fig. 1. One of the main conditions of recording of a suitable excitation spectrum is a nearly linear light emission in the wave-length range requested. For this purpose the DC-generated xenon lamp (Osram XBO 150) proved to be most adapted, which, according to the producer's documentation, has a nearly constant, slightly increasing emission between 330 and 430 nm. The emitted light was monochromated between 377 and 430 nm, using a continuous interference filter (CIF) of Schott, Mainz. The disturbing visible light was filtered out by a combination of BG 38 and BG 3 glass filters. Since, due to the filtration, the intensity of the exciting light was rather low, the emitted light could not be analyzed monochromatically. Therefore, the intensity of the total emission was measured as an indicator of the excitation. A filter type K 510 was applied as the secondary one showing a practically constant transmission above 520 nm.

The relative values of light intensities thus recorded from the galvanometer were corrected for the relative emission values of the XBO 150 xenon lamp according to the producer's documentation, as well as for the relative transmission values of the filters BG 38 and BG 3. In order to achieve a better monochromasy of the exciting light produced by the CIF, a slit-like rectangular measuring diaphragm of 10 × 40 μm size was used.

Recording of the emission spectra. The diagram of the instrument is shown in Fig. 2. Since the recording of the emissions took place between 470 and 650 nm using a K 470 secondary filter, the photometer should have been calibrated for that range. The spectrum dependency of the instrumental sensitivity was measured essentially on the same theoretical basis as performed by SPRENGER and BÖHM [22] with the only difference that the calibration was carried out by means of the XBO 150 xenon lamp, the relative intensities of which were taken from the producer's documentation. During the determination of the correction factor standardized optical arrangements were used (identical diaphragms, magnifications and measuring split-sizes, i.e. 20 × 20 μm). The emitted light was also monochromated by means of CIF. The series of correction factors thus obtained involves, apart from the spectral dependency of the instrumental sensitivity, even the possible wave-length-depending differences in transmittancy of filters K 470 and CIF, as well as to a certain extent the effect of varying dispersion on the measured fluorescence.

To record the emission spectra of the sections, an HBO 200 mercury lamp (Zeiss, Jena) was used. This offered an exciting light of much higher intensity. The exciting light was brought

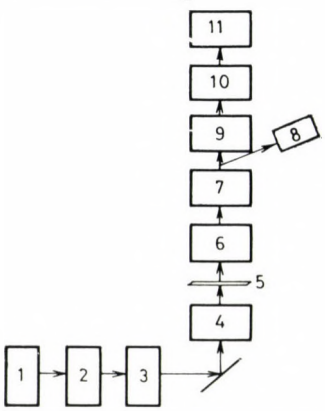

Fig. 2. Schematic diagram of the instrument for recording of emission spectra. 1 = mercury vapour lamp HBO 200 (Zeiss, Jena); 2 = diaphragm; 3 = primary filters: BG 12/2, BG 3/4 (Zeiss, Jena) and HG 405—11 (Schott, Mainz); 4 = glass condenser, n. a : 0.65; 5 = specimen; 6 = objective lens, Pl × 25, n. a.: 0.50; 7 = barrier filter K 470 (Schott, Mainz); 8 = prism for the binocular tube; 9 = projective lens, × 10; 10 = continuous interference filter (CIF) (Schott, Mainz); 11 = photomultiplier tube RCA 1P—21 with light diaphragm

about by means of an Hg 405—11 interference filter (Schott, Mainz). The CIF was placed between the ocular and the measuring split. The recording of a complete emission curve in steps of 10—12 nm took about 2—2.5 min, meanwhile the fluorescence intensity at the maximum decreased by 6—7% on the average.

In order to clear up the origin of fluorophores, the HCl-vapour post-treatment described by BJÖRKLUND and co-workers [1] was also carried out on both paraffinized and deparaffinized sections. The deparaffinization was performed in horizontal position of the sections, the xylene was blotted from the slides, thus the fixation of the sections to the slides with albumin was unnecessary.

In order to achieve the best stability of the light intensities, a stabilized power supply was applied to the lamps.

Results

Excitation spectra

The recorded and corrected excitation spectra for the cerebral and pedal ganglia are shown in Figs 3 and 4. The excitation maximum was at 421 nm, it declined toward the lower wave length, nevertheless, at 400 nm it amounted to 57%. Each value in the excitation curve corresponds to the average of 5

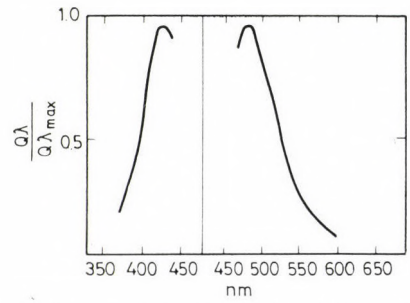

Fig. 3. Cerebral ganglion. Excitation (left side) and emission (right side) curves recorded in the neuropile of the ganglia. The intensities are plotted as relative values vs. wave-length

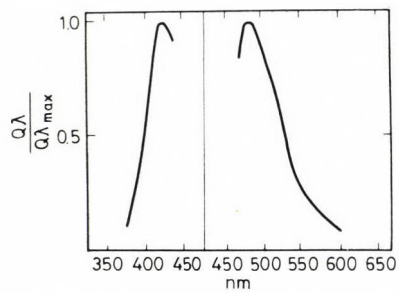

Fig. 4. Pedal ganglion. Explanation, see Fig. 3

measurements. The deviations of the data, being negligible small, were omitted. In the curve the values below 400 nm are obviously lower than the real values since the transmittance of the glass condenser and of the slide declines below this wave length. Being the rate of decline unknown, the curve was not corrected in this sense. However, the decrease in transmittance cannot be high up to 377 nm, since UV-exciation-fluorescence microscopy can be performed with the same condenser and slide and the maximal transmittance of the UG 1 filter used in those cases is at 370 nm.

Apart from the lower values below 400 nm, the excitation curves are similar in shape to that recorded for the CA-containing nerve cells of rat spinal cord by BJÖRKLUND and co-workers [2].

Emission spectra

The recorded and corrected emission curves are also shown in Figs 3 and 4. The measurements were carried out in each of the 5 regions of fluorescing axon groups of both cerebral and pedal ganglia and the data were averaged at each wave-length. Because of their low values the deviations are omitted. The background values without section were recorded at each wave-length and subtracted from the average values of the corresponding emissions. The background values amounted only to 0—3% of the maxima, however, for theoretical reasons they should be taken into correction. The curve thus obtained was corrected by the factors involving the instrumental sensitivity, etc.

The emission maximum is at 482 nm and the emission abruptly decreases towards both directions. At 600 nm it is only 12—13% of the maximal intensity. The shape of the curve is essentially identical with that recorded for the CA-containing nerve cells of rat spinal cord by BJÖRKLUND and co-workers [2].

Results of HCl treatment

The HCl treatment of paraffin sections at room temperature for 1 h caused no remarkable change in the excitation curve. However, on deparaffinized sections even a 1—2-min treatment at room temperature resulted in a moderate shifting of the excitation maximum toward the shorter wave-lengths. Nevertheless, the degree of the shifting was too little even after 4-min treatment, to attribute the fluorophore to norepinephrine [1]. Four minutes or longer treatments were accompanied by the appearance of a strong background fluorescence. On the basis of these investigations the fluorophore can mainly be attributed to dopamine.

Discussion

The essential difference between the method used by us and those mentioned in the Introduction is that we applied CIFs instead of prismatic monochromators. Although the CIF offers a lower degree of monochromasy, it has already been used for spectrofluorometric purposes [4, 10, 20, 21]. Interference filters have also been applied for microspectrofluorometry on the excitation site by MARSDEN and KERKUT [15] as well as by SPRENGER and BÖHM [22].

For the investigation of biogenic monoamines of mast cells a method has been used by VAN ORDEN and co-workers [24] recording 3 points of the excitation and several points of the emission spectra by applying interference filters. Our method can essentially be regarded as a further development of theirs, in so far as the application of CIFs offers a possibility for practically continuous recording of both excitation and emission spectra.

Our method undoubtedly has a disadvantage, namely, that the spectra cannot be recorded as quickly as to avoid any photodecomposition of the fluorophores. Nevertheless, keeping in mind that the photodecomposition amounts to 6—7%, at the maximum,the method can be used. A further disadvantage is the lower sensitivity of the RCA 1P—21 photomultiplier tube as compared with that of the EMI 9558 types used in the more precise instruments mentioned above. This, however, can be disregarded as long as the fluorescence intensity of the object is sufficiently high. If necessary, one can use even more sensitive photomultiplier tubes in our instrument, too. The relatively low sentitivity of our instrument proved to be a limiting factor in our experiments when the recording of excitation spectra could be performed only by measuring the summarized intensity of fluorescence instead of that of the maxima. This, however, did not alter the shape of the curve.

Another disadvantage of our method is the impossibility of automatic recording, since, e.g., the synchronous moving of the CIF with an oscilloscopic recording system would represent an utmost complicated mechanical problem.

In spite of the disadvantages, the method used by us brought results comparable with those obtained by using more complicated, more perfect instruments. Therefore, this method seems to be suitable for qualitative microspectrofluorometric purposes in given excitation and emission wave-lengths. The same method was successfully applied for the analysis of the induced fluorescence of monoamines in insect brain [17].

According to the results, the fluorophores originate in CAs in the neuropile of cerebral and pedal ganglia of *Anodonta cygnea*. This is shown definitely by both the excitation and emission spectra. Among the CAs only the primary ones can come into account, since the parameters of the reaction (1-h formaldehyde treatment) [9] allow us to exclude adrenaline. Distinction between the primary CAs (dopamine and noradrenaline) can be made by means of the HCl treatment and it showed in accordance with the results of biochemical analyses [12] that mainly dopamine was present in the ganglia. This is consistent with the results of other earlier publications [1, 6, 7, 18, 26, 27], too. Consequently, the fluorophores do not contain components of 5HT origin in the areas investigated.

The question arises, where the 5HT can be localized in the neuropile of the ganglia. It seems to be sure that the cytoplasm of the ganglion cells contains 5HT [6, 7, 26]. It is also sure that relatively large amounts of 5HT are present in the cerebrovisceral connective (CVC), nearly 50% of that of the cerebral ganglion [11]. Notwithstanding, the Falck—Hillarp technique failed to reveal 5HT in the CVC (own unpublished result). Since the nerve fibres of the CVC originate in the cerebral ganglion, at least in a great part, the 5HT must come from the ganglion cells into the CVC. However, in this case the 5HT-containing fibres have to cross the neuropile of the ganglion. On the other

hand, the axonal presence of 5HT is also indicated by the results obtained by means of differential and density-gradient centrifugation [13]. Consequently, apart from the CAs, the presence of 5HT fluorophores should also have been detected in the neuropile.

According to the measurements of MÖLLMANN and co-workers [16], the emission curve is characteristically "two-humped" in the cells of the glomus caroticum of rabbits where CAs and 5HT occur together, and this can be derived mathematically from the summation of the emissions of two types of fluorophores. Since the 5HT fluorophore shows maximum at 540 nm, we should have recorded a second maximum at this place even in *Anodonta*.

According to quantitative analyses, the μg/g wet-weight concentrations of 5HT are more than 5 times higher in the cerebral and more than 3 times higher in the pedal ganglia of *Anodonta cygnea* than the summarized concentrations of dopamine and noradrenaline [11, 12]. Since the molecular weights of these 3 compounds (dopamine = 153, noradrenaline = 169, 5HT = 176) are near to each other, their molar concentrations differ from each other nearly to the same degree. In model experiments the dopamine and noradrenaline resulted in about 3 times higher fluorescence intensity than the same molar concentration of 5HT [14]. Considering these data, one should expect that in case of uniform intraganglionar distribution of the monoamines the relative intensity of fluorescence at the maximum of 5HT (540 nm) is about 1.6 times higher than that observed at the CA maximum (480 nm) in the cerebral ganglion, whereas in the pedal ganglion the intensity is approximately the same at these two wave-lengths. Even if the quicker photodecomposition of the 5HT fluorophore [14] is taken into consideration, the complete absence of the emission maximum characteristic of 5HT can only be explained by assuming either the absence of 5HT from the neuropile or the failure of the formation of the fluorophore from it. The same information was obtained from the excitation spectra: if 5HT fluorophore is present, one should find an excitation maximum at 380 nm; however, this was not the case.

Considering all these, one can hardly accept the absence of 5HT from the neuropile. DAHL and co-workers [6] described structures assumed to be the transversal sections of 5HT-containing axons, however, these structures proved to be gliosomes during the histological identification in the neuropile of *Anodonta cygnea* [26]. Therefore, one can assume that the 5HT is present in the neuropile either alone or together with CAs, however, in both cases it may be indetectable by the routine Falck—Hillarp technique.

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Acta biol. Acad. Sci. hung., **24** (3—4), 201—205 (1973)

PREVENTION BY CYCLOHEXIMIDE OF CELLULAR AUTOPHAGY INDUCED BY HYPEROSMOTIC SUCROSE OR CADMIUM CHLORIDE IN MOUSE PANCREATIC ACINAR CELLS

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(Received 1973—06—11)

Abstract

Formation of autophagic vacuoles was induced in mouse pancreatic acinar cells by single intraperitoneal injection of either cadmium chloride (1.5 mg/animal) or a hyperosmotic solution of sucrose (15 per cent w/v, 1 ml/animal). The animals were killed 3 h later. This reaction was prevented by cycloheximide (0.2 mg/g b. w.) given 30 minutes prior to the injection of either damaging agents. This was a transient protection, since autophagic vacuoles developed in the cells under the effect of the inducers given 24 h after the administration of the drug. While cycloheximide alone caused no considerable morphological alteration in the cytoplasm in short-term experiments, a sporadic occurrence of autophagic vacuoles was characteristic of the cells 24 h after the treatment. These results provide further evidence that cycloheximide is capable of preventing induced autophagocytosis, regardless of the different chemical nature of its inducer.

Introduction

Recently, we have reported that cycloheximide pretreatment prevented the neutral red-induced formation of autophagic vacuoles in a number of cell types, *viz.*, pancreatic exocrine [6, 11], seminal vesicle epithelial [6], and hepatic parenchymal [6] cells of the mouse, as well as in chicken pancreatic [6,] and liver [6] cells. As it was found ineffective [1, 12] on glucagon-induced autophagocytosis in rat liver cells, in the present work we attempted to demonstrate protective action of this drug against autophagy induced by agents other than neutral red. This paper reports the results of experiments in which treatments with either hyperosmotic sucrose or Cd^{++} ions, agents known to induce autophagocytosis [10], were preceded by injections of the antibiotic.

Material and method

Forty-five male CFLP mice weighing 30 g each were used. Cycloheximide (Sigma) was dissolved in distilled water immediately before use to make 6 mg/ml solution. The mice were given a single non-lethal dose (0.2 mg/g body weight) of the drug by intraperitoneal injection. The sensitivity of mice to cycloheximide seems to be strain-dependent, since we found the lethal dose higher in CFLP mice than in an inbred strain used earlier [11]. Animals exposed to cadmium or sucrose were injected intraperitoneally with 0.3 ml of an 0.5 mg/ml

aqueous solution of cadmium chloride and with 1 ml of a 15 per cent w/v sucrose solution, respectively. The experiment was scheduled as follows.

Group 1. Ten mice were given cycloheximide, and decapitated 210 min or 24 h later, five animals at each time.

Group 2. Five mice were injected with sucrose and killed after 3 h.

Group 3. Five animals received cadmium chloride and were sacrificed 3 h later.

Group 4. Ten mice received cycloheximide and 30 min or 24 h thereafter were injected with cadmium chloride, 5 animals at each time. All were decapitated 3 h after the cadmium treatment.

Group 5. Five animals were given the sucrose injection 30 min, and another 5, 24 h after the pretreatment with cycloheximide. They were killed 3 h after the sucrose treatment.

Group 6. Five animals given 1 ml distilled water intraperitoneally and killed 3 h later served as controls.

Small pieces of the pancreas were fixed in distilled, 3% glutaraldehyde (Fluka) buffered with pH 7.4 (0.125 mol/l) phosphate buffer, for 2 h, postfixed for 1 h in 2% osmium tetroxide and embedded in Araldit. A UEMV—100B electron microscope was used for the investigations.

Results

As it was expected on the basis of the results of an earlier detailed investigation of the phenomenon [10], autophagic vacuoles containing mainly degrading cisternae of the rough-surfaced endoplasmic reticulum were observed in the acinar cells 3 h after the injection of either cadmium chloride

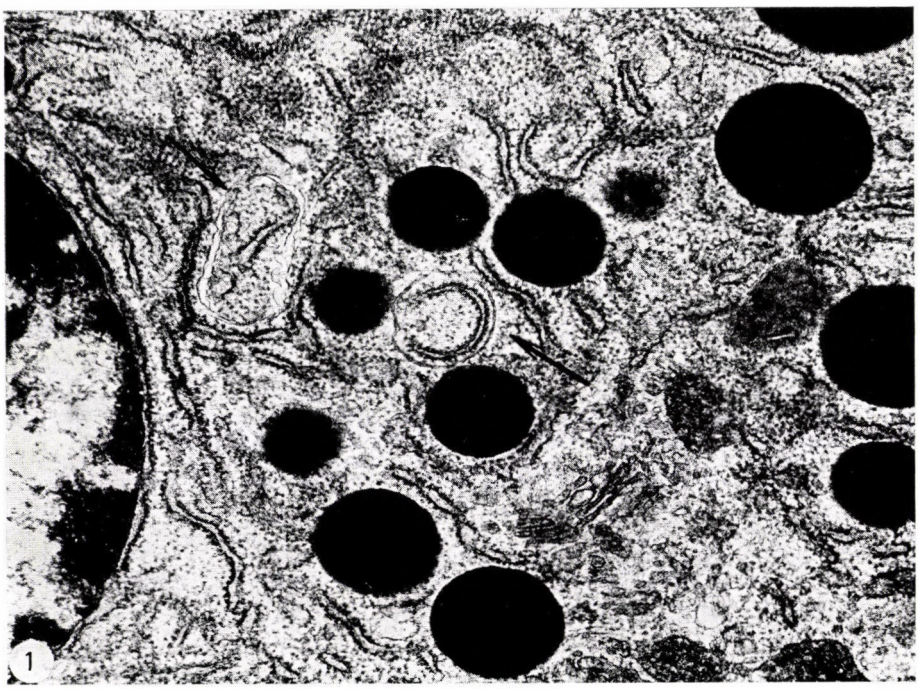

Fig. 1. Part of pancreatic acinar cell from a mouse 3 h after cadmium-chloride treatment. Early forms of double membrane-limited autophagic vacuoles with rER cisternae encapsuled are indicated with arrows; × 25 000

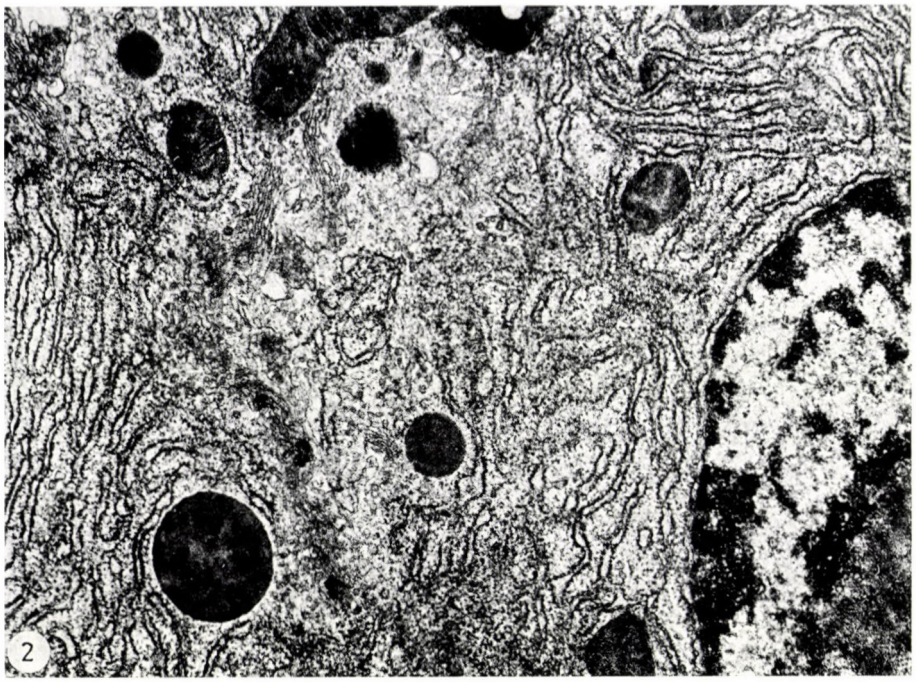

Fig. 2. Part of pancreatic exocrine cell from an animal given cycloheximide 30 minutes prior to cadmium chloride for 3 h. No autophagic vacuoles are present; × 21 000

(Fig. 1) or sucrose. The autophagic vacuoles were found most abundantly in the vicinity of the Golgi zone, and their morphology corresponded to that described earlier [10].

On the other hand, no induction of autophagocytosis by either cadmium chloride (Fig. 2) or sucrose was observed in the cells of mice given cycloheximide 30 min prior to the inducer. The cytoplasmic structure of these cells could not be distinguished from those treated with cycloheximide alone for 210 minutes. Apart from the apparent deletion of zymogen granules and a hypoplasia of the Golgi apparatus, the drug itself caused no considerable cytoplasmic alterations within this time.

When sucrose or cadmium were administered to the mice 24 h after cycloheximide, numerous autophagic vacuoles were seen in the cells 3 h after the administration of the inducer (Fig. 3). Moreover, a small number of autophagic vacuoles were present in the cytoplasm 24 h after the injection of cycloheximide (Group 1).

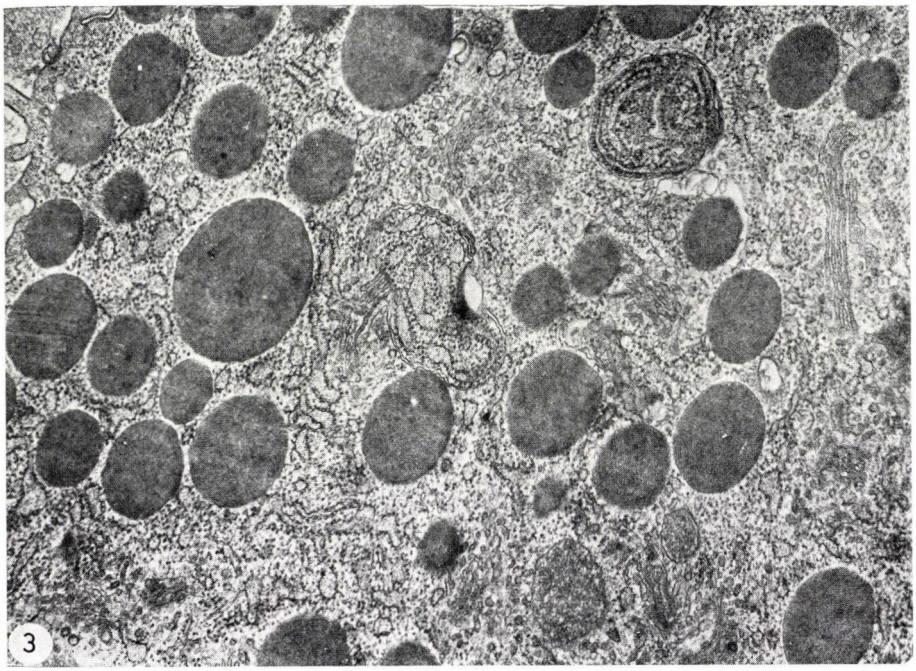

Fig. 3. Autophagic vacuoles are evident in an acinar cell from a mouse given cycloheximide 24 h prior to sucrose for 3 h; × 18 000

Discussion

Based on the results presented here, and on previous experiments in which neutral red [6, 11] or puromycin [7] was used as inducer of autophagocytosis, we conclude that cycloheximide given in lethal or sublethal doses to animals is capable of preventing induced autophagocytosis in the pancreas. This is a transient effect since the cells recovered their capability for autophagic response to the injurious agents within 24 hours. Our previous, partly published findings indicate that the length of the interval during which cycloheximide prevents focal cytoplasmic degradation is directly proportional to the dose of the drug.

Based upon our earlier results [10], it is obvious, that in pancreatic acinar cells the rough-surfaced endoplasmic reticulum may serve as a major source of the limiting membrane of the autophagic vacuoles.

Since other translational inhibitors like dimethylnitrosamine [4], puromycin [7, 8] and ethionine [2, 5] are known to induce autophagocytosis and a degranulation of the endoplasmic reticulum as well, we hypothesized [11] that cycloheximide might act as an inhibitor of a membrane transformation necessary [3] for the formation of the autophagic vacuoles, possibly

through its preserving effect on membrane-bound polyribosomal structure. Experiments are being carried out in our laboratory on prevention of induced autophagy by emetine, a translational inhibitor acting similarly as cycloheximide [9].

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Acta biol. Acad. Sci. hung., **24**(3—4), 207—214 (1973)

OBSERVATIONS ON MAMMALIAN PANCREATIC CELLS GROWN IN MONOLAYER CULTURES

V. QUANTITATIVE STUDY OF THE NUCLEOLAR EXTRUSION IN RAT PANCREATIC CELLS CULTIVATED IN VITRO

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(Received 1973—07—09)

Abstract

Attempts were made to analyse quantitatively and to influence nucleolar extrusion which is visible under the light microscope.The experimental model consisted of insulin-releasing rat pancreatic cells cultivated *in vitro*. Attempts to increase the nucleolar extrusion by treatment with various substances were unsuccessful. No correlation was found between insulin release and nucleolar extrusion. The process of nucleolar extrusion was examined by micro-cinematography, in stained preparations and by electron microscopy using the Stereoscan®.

Introduction

In our first paper on the pancreatic cells of adult rats cultured as monolayers [4], nuclear alterations including the extrusion of nuclear substance and whole nucleoli through openings of the nuclear membrane were described. We have interpreted this process — like HSU and LOU [7] and ALTMANN [1] — as the manifestation of an extremely intense metabolism of the cells concerned, which is characterized by production and secretion phases. The question was raised whether the extrusion of nuclear substance and the insulin release are connected phenomena. In the present work the course and the quantitative distribution of the extrusion of nuclear material were followed in experiments in which the insulin release of *in vitro* cultivated pancreatic cells was influenced experimentally.

Material and method

Pancreases of male rats of 40 g body weight were used throughout. The preparation of cell suspensions and the cultivation techniques have been described elsewhere [4].

Nucleolar substance extrusion (NSE) was examined quantitatively from the 3rd to the 12th day of cultivation *in vitro*, in May—Grünwald—Giemsa preparations. Each value is based on the examination of 3 000 cells. Analyses of individual pictures sorted out from the micro-cinematographic photographs showed details of the NSE, especially that of the nucleolar extrusion. Attempts were made to influence this process by certain substances (caffeine, glucose, pilocarpine, colchicine, bile acids and sulphonyl derivatives of urea) on the 5th day.

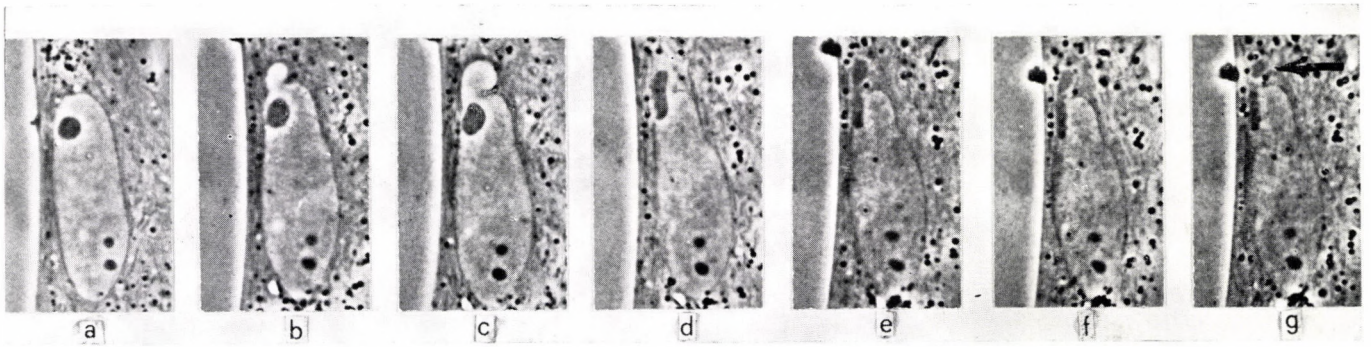

Fig. 1. Process of the nuclear substance extrusion including nucleolar budding-off (time-lapse film). a—c = formation of a nuclear bleb; d—g = the budding-off of a nucleolus-portion which remains in the cytoplasm (→). × 900

Results

The NSE, as it was followed by microcinematography, is presented in Fig. 1. A slowly growing weakly structured nuclear bleb folds out and an elongated nucleolus wanders into it. By splitting up of the bleb the nuclear membrane opens. Thereafter, a portion of the nucleolus detaches, and in the cytoplasm, slowly loses its contrast. The nucleolar material remaining within the nucleus either takes a position beside the nuclear membrane having been restituted in the meantime, or wanders, as a rounded particle, towards the inner region of the nucleus. Nuclear blebs can repeatedly be formed in short

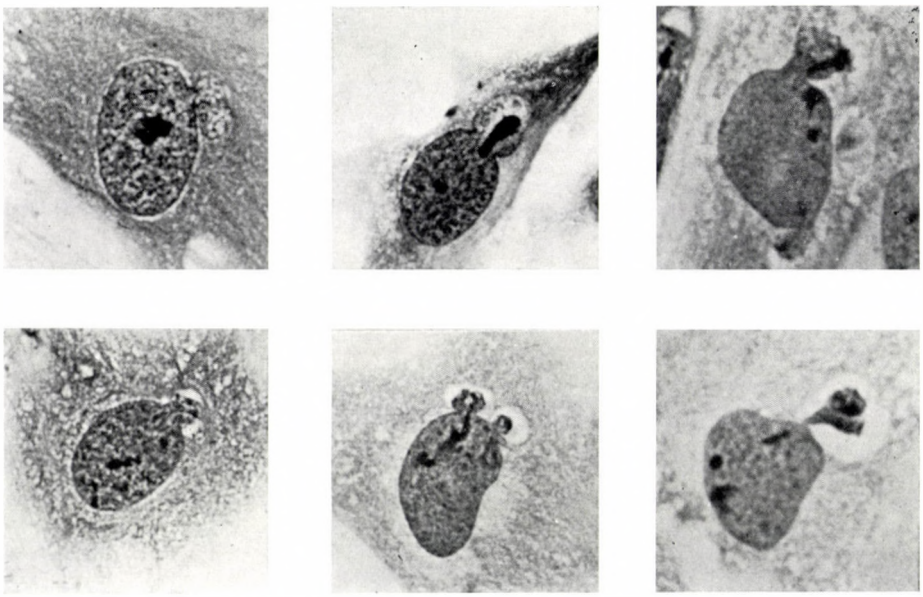

Fig. 2. Different stages before, during and after the nuclear substance extrusion. May—Grünwald–Giemsa staining. Magnification: about × 800

periods. Nucleoli do not wander into all of these blebs. Nevertheless, the nuclear membrane opened and the blebs split in all the cases observed by us. After restitution of the nuclear membrane a vacuole develops at the place of the former opening. Extrusion of the nucleolar material takes place in a few seconds or minutes, whilst the so-called prephase and postphase can last hours. In the quantitative evaluation based on stained preparations we divided the NSE into three phases:

prephase: formation of the bleb and immigration of the nucleolus;

output phase: opened nuclear membrane and extrusion of a nucleolus (or a portion of the nucleolus) in some of the blebs;

postphase: restituted nuclear membrane and a vacuole formed at the place of the former opening.

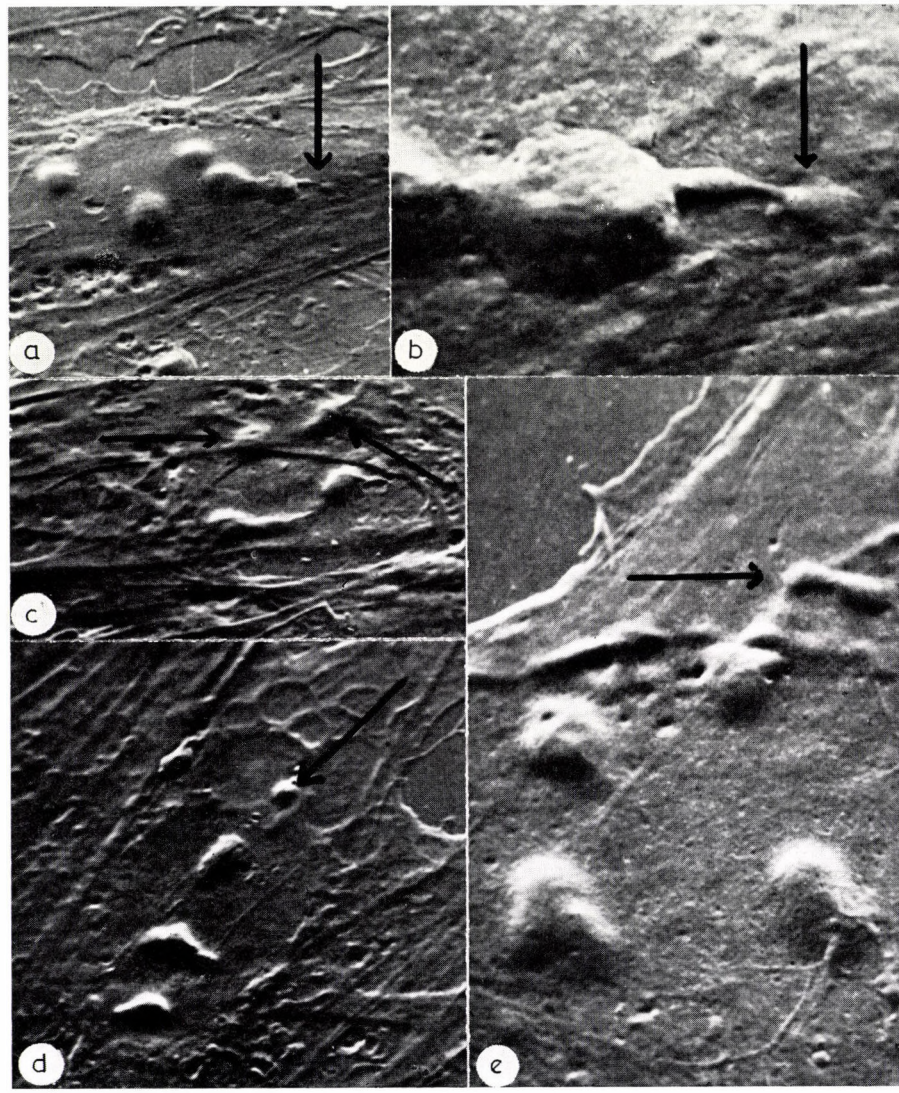

Fig. 3. Extrusion of nucleolar portions taken up with the scanning electron microscope. a and b = budding-off and extrusion (→) of nucleolar portions. Magnifications: a = × 2 400; b = × 12 000. c—e = nucleolar portions outside of the nucleus (→). Magnifications c and d = = × 2 400, e = × 6 000

Fig. 2 shows a few stages of this process in May—Grünwald—Giemsa preparations. In Fig. 3 taken with the scanning electron microscope, the nucleolar extrusion can be seen.*

* The photographs were made in the laboratory of Dr. SCHERER. I express my thanks to Mrs. SCHMIDT for the good collaboration, working with the Stereoscan®.

Fig. 4 presents the frequency of the NSE in the first passage *in vitro*. Depending on the growth intensity, it increases markedly in the first days. The maximum frequency was reached as a rule on the 7th day, 6 and 10 days being the extreme values. In the two hours following changing of the medium the process becomes more frequent again.

We examined the effect of glucose, tolbutamide, glybenclamide, secretin (pure and commercial), insulin, pilocarpine and different bile acids. Significant increase in the number of NSE, as it was observed by ALTMANN [1] *in vivo* in exocrine mouse pancreatic cells after pilocarpine, was found in only one of our numerous experiments.

NSE was suppressed by caffeine (Fig. 5), by colchicine (Fig. 6) and by prolonged treatment with insulin or commercial secretin. Numerous combined experiments in which two substances were given one after another or simultaneously for two hours showed the following:

(a) the inhibition of the NSE by colchicine is irreversible (Fig. 6); it cannot be suspended by either of normal nutritive medium, pure secretin, caffeine and insulin;

(b) in one experiment, glybenclamide (1 μg/ml) in combination with either of the three bile acids (10μg/ml) led to an activation of the NSE. In similar combinations pure secretin, failed to increase the poor effect of the bile acids (Fig. 7).

Discussion

Lightmicroscopically observable extrusion of nuclear material, occasionally of whole nucleoli, has often been described in the last 20 years. In some of these works tissue sections were studied. ALTMANN [1] and CLARK [2], among others, have analyzed exocrine pancreas tissue and SCHMALBRUCH [10] human skeletal muscle, the two latter authors have evaluated electron micrographs. NSE in cells cultivated *in vitro* was studied, e.g., by POMERAT and co-workers [9], HSU and LOU [7] and LOVE and co-workers [8] who had evaluated optical and electron micrographs and partly used the microcinematography. More recently, GODINA and co-workers [3] have described the extreme case, namely, the extrusion of the whole nucleolus and its disappearance within the cytoplasm. The extrusion of nucleoli or of parts of them as observed by us, proceeds similarly to that described by HSU and LOU [7] in melanoma cells, by LOVE and co-workers [8] in permanent cell lines and by GODINA [3] in chicken heart fibroblasts. While HSU and LOU as well as GODINA worked with uninfluenced cells, LOVE and co-workers succeeded — in contrast with our experiments — in stimulating the extrusion of nuclear material (non-physiological temperatures, 15'/46°C). The stimulation was measured by the increase in the mass of the nuclear material found outside the restituted nuclear mem-

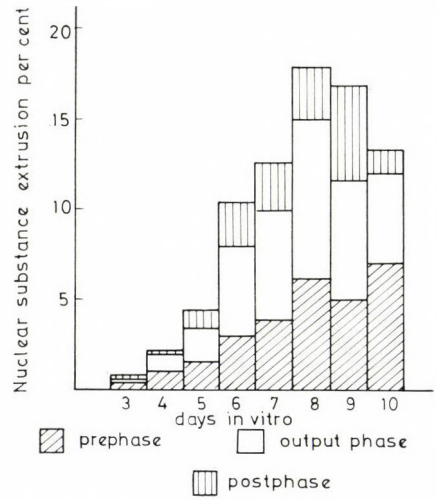

Fig. 4. Frequency of the nuclear substance extrusion in the first passage *in vitro*

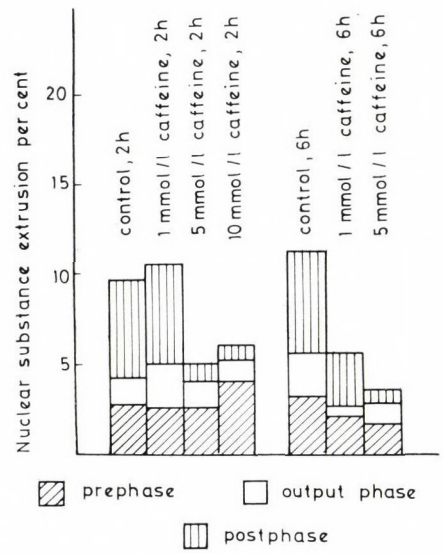

Fig. 5. Effect of caffeine (1, 5 and 10 mmol/l) on the nuclear substance extrusion

brane. The extruded mass was identified chiefly as DNA-containing nucleolar material. In our experiments the extruded parts were Feulgen-negative.

In our experiments, in general, NSE was inhibited, independently of the effect exerted by the substance on the insulin release (e.g., increase by caffeine, no effect by colchicine; HILWIG [5, 6]).

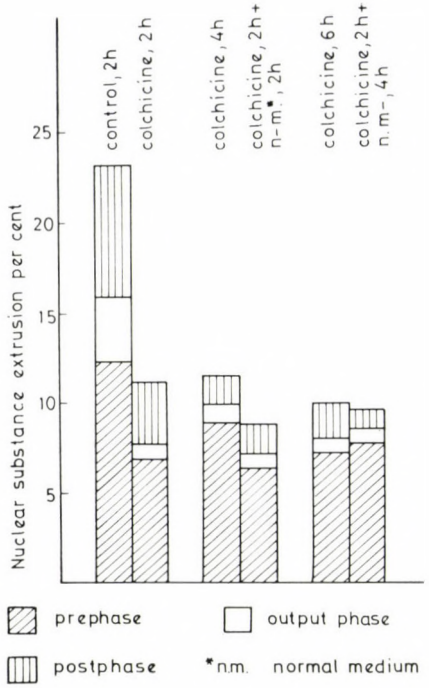

Fig. 6. Effect of colchicine (1 μg/ml) on the nuclear substance extrusion

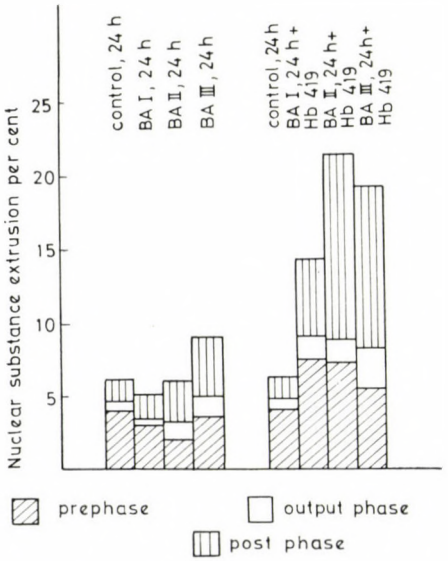

Fig. 7. Effect of glybenclamide (1 μg/ml, HB 419) in combination with bile acids BA I—BA III (10 μg/ml) on the nuclear substance extrusion. BA I = taurodeoxycholic acid; BA II = taurochenodeoxycholic acid; BA III = taurolithocholic acid

Only one of the experiments has led to an increase of the NSE. This was performed in a cell strain showing extrusion stages at a low frequency. Here the NSE was stimulated by glybenclamide, in combination with either of the bile acids, under study (Fig. 7). The specific or nonspecific character of this stimulating effect has not been cleared up. Parallel examinations, for example, with pure secretin combined with bile acids, showed no increase in the NSE.

Our experiments pointed out that the NSE and the insulin release have no connection with each other.

Acknowledgement

I thank Mrs M. Vöth, Miss E. Leschinski and Mr M. Meisinger for their precious collaboration in performing the examinations.

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Acta biol. Acad. Sci. hung., **24** (3—4), 215—220 (1973)

MOSAIC CENTROMERIC FUSION IN A HOLSTEIN-FRIESIAN BULL

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(Received 1973—08—03)

Abstract

In the PHA-stimulated peripheral blood cultures of a four-year-old Holstein-Friesian bull without any phenotypical abnormality two cell lines were found. The majority of mitoses had a completely normal male karyotype with 60 chromosomes. Three analyzed divisions out of 36, however, had only 59 chromosomes with one long metacentric form. One cell had 60 chromosomes including this anomalous form. The metacentric presumably takes its origin from centromeric fusion of two medium-size autosomes. This chromosomal anomaly may be responsible for the low fertility of the sire in spite of excellent laboratory quality of his sperm.

Introduction

The Robertsonian translocation or centromeric fusion is one of the most frequent autosomal anomalies described in cattle. A number of papers have dealt with the translocation of one or both chromosomes [1—6, 9—15, 21—23, 27, 28] No. 1 to No. 29, i.e. No. 1 to No. 28 [24]. Centromeric fusion of chromosomes No. 2 to No. 4 was reported, too [20]. The translocations may be heterozygous or homozygous, whether one member or both of the chromosomal pairs are involved.

This type of translocation is readily recognizable by the presence of one or two long metacentric chromosomes beside one pair of metacentric sex chromosomes and uniquely acrocentric autosomes.

Another type of autosomal fusion is the so-called "tandem" translocation. A part of one chromosome arm is translocated to the distal part of another and it results in a long acrocentric chromosome [7, 8].

The normal diploid number of the cattle is 60 [17, 18]. This number is reduced to 59 in case of heterozygous and to 58 in case of homozygous translocation. These chromosomal anomalies usually do not influence the phenotype of the cattle. Nevertheless, the chromosomal anomaly of a sire may cause reduced fertility [7, 8]. The same effect was established in the heifers carrying the anomaly [3].

Material and method

The propositus is a four-year-old Holstein-Friesian bull, homozygous for the red-white colour, imported from Canada. The animal is phenotypically normal without any pathological signs. Haematological findings were normal by repeated examinations. Though his sperm turned out to be of impeccable quality by laboratory investigations, his fertility has been low: 37.1% out of 1 162 first inseminations in comparison with a mean 50.3% among other sires used on the same farms.

Chromosomal specimens were obtained by cultivating PHA-stimulated peripheral venous blood during 72 h at 37 °C according to MOORHEAD and co-workers [19]. Four μg/ml Colcemid was added to the cultures four hours before the harvest. The slides were stained for chromosomal bands by the method of SUMNER and co-workers [25] with a modification used in our laboratory [26]. The well-spread mitoses were photographed and enlarged. The chromosomes were cut out from prints of the best mitoses and ordered according to their size and bands, provided the latters were recognizable.

Results

A total of 36 mitoses were analyzed out of two cultures of peripheral WBC.

Four cells contained a long metacentric chromosome. Its size is comparable with that of the X chromosome, the latter being more submetacentric. Out of these, three cells had 59 (Fig. 1) and one cell had 60 chromosomes (Fig. 2).

Sixteen cells possessed 60 chromosomes with a completely normal karyotype (Fig. 3).

Thirteen further mitoses had less than 60 chromosomes, but they lacked the translocated form (Table 1). In these cells the loss of the chromosomes was random.

Three mitoses, excluded from Table 1, had approximately tetraploid counts, but they were not unequivocally analyzable for the presence of the translocated form. No other structural rearrangements were found.

Table 1

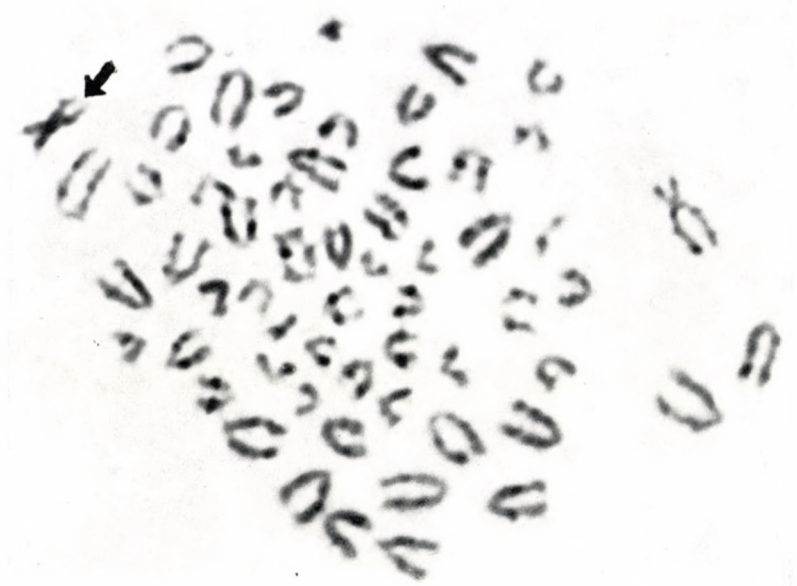
*Chromosome number and occurrence of translocation in diploid mitoses**

Chromosome number	<59	59	60	Total
Number of cells without translocation	8 (24.2%)	5 (15.2%)	16 (48.5%)	29 (87.9%)
Number of cells with translocation	— —	3 (9.1%)	1 (3.0%)	4 (12.1%)

* Three tetraploid mitoses are not included in this table

Discussion

In the case presented here an abnormal cell line was found beside the normal karyotype in the peripheral blood cultures of a bull. The anomaly is represented by a long metacentric chromosome. We assume that it was pro-



a)

b)

Fig. 1a—b. a = mitosis with 59 chromosomes, one of them being the translocated form (arrow); b = karyotype made of the same division. The anomalous metacentric chromosome placed outside right. Autosomes Nos 13 and 21 left unpaired. Tentatively they took part in the centric fusion

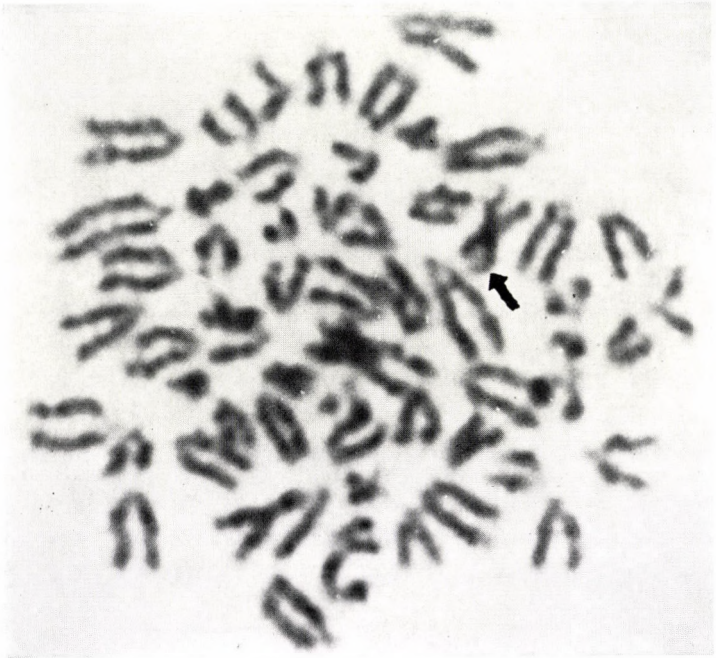


Fig. 2. Another mitosis with 60 chromosomes, one of them is the translocated metacentric chromosome (arrow)

duced by centromeric fusion of two medium size autosomes. The exact serial number of chromosomes involved in the translocation could not be unequivocally established even by Giemsa band technique, but certainly they do not correspond to the pairs No. 1 and 29, or 2 and 4, described by others as taking part in centric fusion in the cattle. The long metacentric chromosome occurred in cells with 59 chromosomes. Only one cell possessing this anomaly had a normal diploid count, i.e., 60 chromosomes. This fact speaks for centric fusion laying in the basis of this anomaly, found in 12.1% of the analyzed divisions. The centromeric fusion is of heterozygous kind, for the other two members of the corresponding pairs remained uninvolved.

Phenotypically the bull does not reveal any sign prescribable to the chromosomal anomaly. However, his low fertility may be connected with the mosaic anomaly of his chromosomes. Further investigations of the offsprings are in progress. On the other hand, some exogenous mutagenic influences might also cause the described chromosomal anomaly.

Acknowledgements

We wish to thank Miss KATALIN G. MOLNÁR and Miss ÉVA BORDÁS for skillful assistance.

Fig. 3. Normal male division and karyotype (60 chromosomes) of the same sire

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Acta biol. Acad. Sci. hung., **24**(3—4), 221—231 (1973)

TRANSNEURONAL EFFECTS IN THE DEVELOPMENT OF THE ADRENERGIC PERIPHERAL INNERVATION APPARATUS

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(Received 1973—09—25)

Abstract

In contrast to the resistance of the peripheral adrenergic innervation apparatus to preganglionic denervation (decentralization) in adult animals, striking alterations were observed in the fluorescence microscopic pattern of the iris in suckling rats subjected to preganglionic transection of the cervical sympathetic trunk. Decentralization performed before the 7th postnatal day results in a considerable retardation of postganglionic structures; the same operation if performed during the second postnatal week, i.e., in the "critical period" of the development of sympathetic ganglion cells in the superior cervical ganglion, results in a luxuriant hyperproliferation of postganglionic structures. Decentralization performed after this period does not induce any alterations in the postganglionic innervation apparatus. Irrespective of the effects upon the postganglionic structures, decentralization performed during the first two postnatal weeks results in a survival and proliferation of the "spherical bodies", characterizing early developmental stages of the postganglionic adrenergic innervation apparatus. *In vivo* and *in vitro* experiments performed with various depletor and liberator substances suggest that spherical bodies contain serotonin, concentrated in special tissue mast cells that appear to take part in the structural and functional maturation of the autonomic ground plexus.

Introduction

Biochemical investigations [1—3, 7, 10, 12, 20] have proved that catecholamine contents of adrenergically innervated tissues decrease after postganglionic denervation. Also fluorescence microscopic studies performed on the rat iris after removal of the superior cervical ganglion [8, 9, 13—16] have shown disappearance of the specific catecholamine reaction characterizing the normal innervation apparatus.

It is generally assumed, however, that, in striking contrast to the depletion of transmitter stores after denervation, resulting in degeneration of the peripheral innervation apparatus, decentralization, i.e., preganglionic transection does not induce any major alterations in the histochemical structure of the adrenergic nerves in general and their catecholamine content in particular [8, 12, 17].

All this information was obtained, however, on adult animals. Thus the question arises whether independence of the peripheral innervation apparatus from the preganglionic neurone holds also for early stages of ontogenesis, viz.,

during the very development of the adrenergic ground plexus. We *aimed* at answering this question, which seems to be fundamental for understanding the dynamics of autonomic neurogenesis.

Material and method

Investigations were performed on 56 albino rats aged 1—30 days.

The cervical sympathetic nerve was transected one millimeter below the superior cervical ganglion. Thus we obtained decentralization of the autonomic innervation apparatus of the dilatator muscle in the iris. Sympathetic ganglion cells whose axons constitute the ground plexus innervating the dilatator muscle are located within the superior cervical ganglion. These ganglion cells receive preganglionic fibres *via* the cervical sympathetic trunk from the ciliospinal centre located in the spinal segment Th 1.

Using a Zeiss binocular operating microscope, the above surgery was performed in suckling rats on the 2nd, 4th, 6th, 8th, 10th, 12th, 14th, 17th and 21st postnatal days under Nembutal anesthesia. Rats were killed on the 2nd, 4th, 7th, 10th, 14th, 21st and 25th postoperativ days. Irides of both sides were dissected in saline, stretched on non-fluorescent microscope slides, treated with formaldehyde gas according to the technique of ERÄNKÖ, FALCK and HILLARP and studied under a Zeiss fluorescence microscope, using a HBO 200 W OSRAM high-pressure mercury lamp, a BG 12 exciter filter and an OG 1 ocular filter. Irides of the nonoperated side served as controls. Photomicrographs were obtained on ORWO 27 DIN black-and-white film.

After the fluorescence microscopic investigation, the preparations were post-stained with toluidine blue and, using the nonius stage of the microscope as a reference system, areas studied fluorescencemicroscopically were re-photographed in visible light.

Results

Development of the adrenergic ground plexus of the rat iris continues after birth; more properly, catecholamine content of the adrenergic ground plexus reaches a histochemically demonstrable level only postnatally. Adrenergic terminals show well-defined varicosities at the 8th postnatal day; arrangement of the varicosities becomes regular at the end of the second postnatal week. In three-week-old rats the fluorescence microscopic pattern of the iris is already identical with that of the adult animal.

Denervation (i.e., removal of the superior cervical sympathetic ganglion) results in all stages of development in a complete disappearance of the fluorescence of the autonomic ground plexus, in full accordance with the obligatory degeneration of postganglionic nerve fibres after removal of their parent cells located within the ganglion.

In striking contrast to the above observations, decentralization (i.e., preganglionic denervation) resulted in entirely different effects when performed in early or later stages of development. Decentralization performed soon after birth (2nd, 4th or 6th postnatal days) resulted in a retardation of development of postganglionic structures (Figs 1a and 1b). The difference between control and decentralized irides was most evident on the 25th postnatal day. In this period, the control side exhibits the pattern of the varicose ground plexus

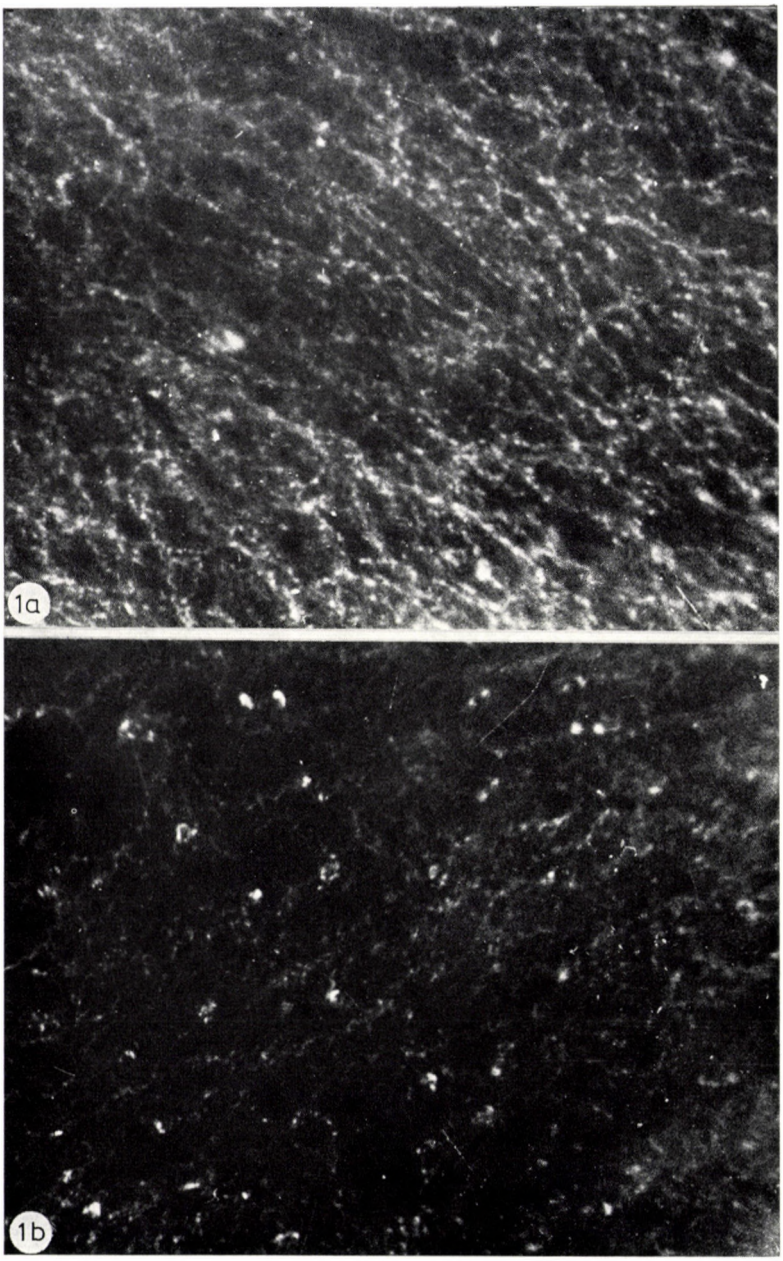

Fig. 1. Fluorescence microscopic pattern of the rat iris on the 25th postnatal day. a = control; b = preganglionic denervation on the 4th postnatal day. Note the loose, immature ground plexus, containing numerous "spherical bodies"; × 250

characterizing adult animals, whereas in the decentralized iris only a loose, less ripe plexus can be seen, characterizing earlier stages of development. Persistence of "spherical bodies", characterizing early stages of development of the autonomic nerve plexus [5] is invariably observed in such specimens; the interpretation of this peculiar situation will be dealt with in detail later.

On the other hand, if the preganglionic denervation, i.e., decentralization, was performed in a later stage of development, i.e., on the 8th or 10th postnatal day, the operated side offered a conspicuously more dense innervation apparatus than the control (non-operated) side. (Figs 2a and 2b). A more thorough inspection reveals, however, that this seemingly "hyper-innervated" structure consists of a great amount of immature axons.

Decentralization performed after the 14th postnatal day did not induce any change in the fluorescence microscopic pattern of the innervation apparatus.

As mentioned above, spherical bodies characterize early stages of development of the autonomic ground plexus. These peculiar fluorescent bodies, located within the cytoplasms of a special cell type, were observed in the irides and other autonomically innervated organs soon after birth. In the course of normal development, these spherical bodies continue to be present until the second postnatal week, though in a slowly decreasing number. After the completion of the formation of the autonomic ground plexus, spherical bodies disappear. We found a striking survival of these spherical bodies in irides subjected to an early decentralization, i.e., through the first week of postnatal life.

In order to identify the cytochemical characteristics of the spherical bodies, various staining procedures and depletion experiments were performed. Formaldehyde-induced fluorescence is confined to intensely reacting granules within the cytoplasms of rounded or ovoid cells (Fig. 3). Yellow colour of fluorescence and photosensitivity suggests their content to be serotonin rather than a catecholamine. This assumption is supported also by *in vivo* and *in vitro* experiments (depletion, accumulation and histamine liberation, Table 1).

Of the drugs used in the present experiments, reserpine is known to deplete catecholamines and serotonin from tissue stores by blocking the uptake-storage in the amine granules, while α-methyl-*m*-tyrosine depletes only catecholamines, by inhibiting their biosynthesis. Compound 48/80, Polymyxin B — histamine-liberator substances — cause degranulation in the mast cells and release the stored serotonin with histamine liberation simultaneously.

Cells containing fluorescent spherical bodies were identified by post-staining with toluidine blue. It was shown that, in effect, we are dealing with tissue mastocytes, being in a peculiarly close correlation with the developing adrenergic ground plexus. Using their metachromatic reaction for identification, it turned out that, as contrasted to control samples, in irides subjected

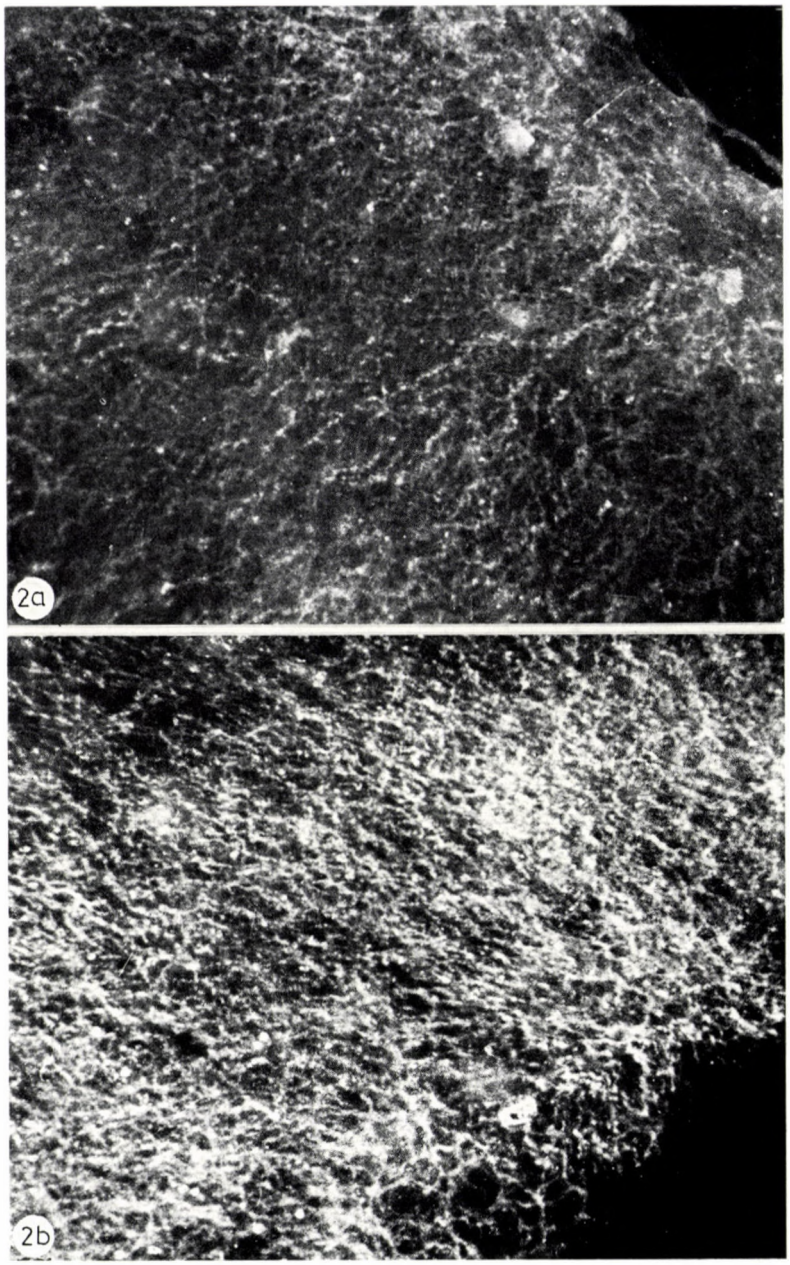

Fig. 2. Fluorescence microscopic pattern of the rat iris on the 25th postnatal day. a = control; b = preganglionic denervation performed on the 10th postnatal day. Note the luxuriant hyperproliferation of immature axons, resulting in a conspicuously "dense" innervation pattern; × 120

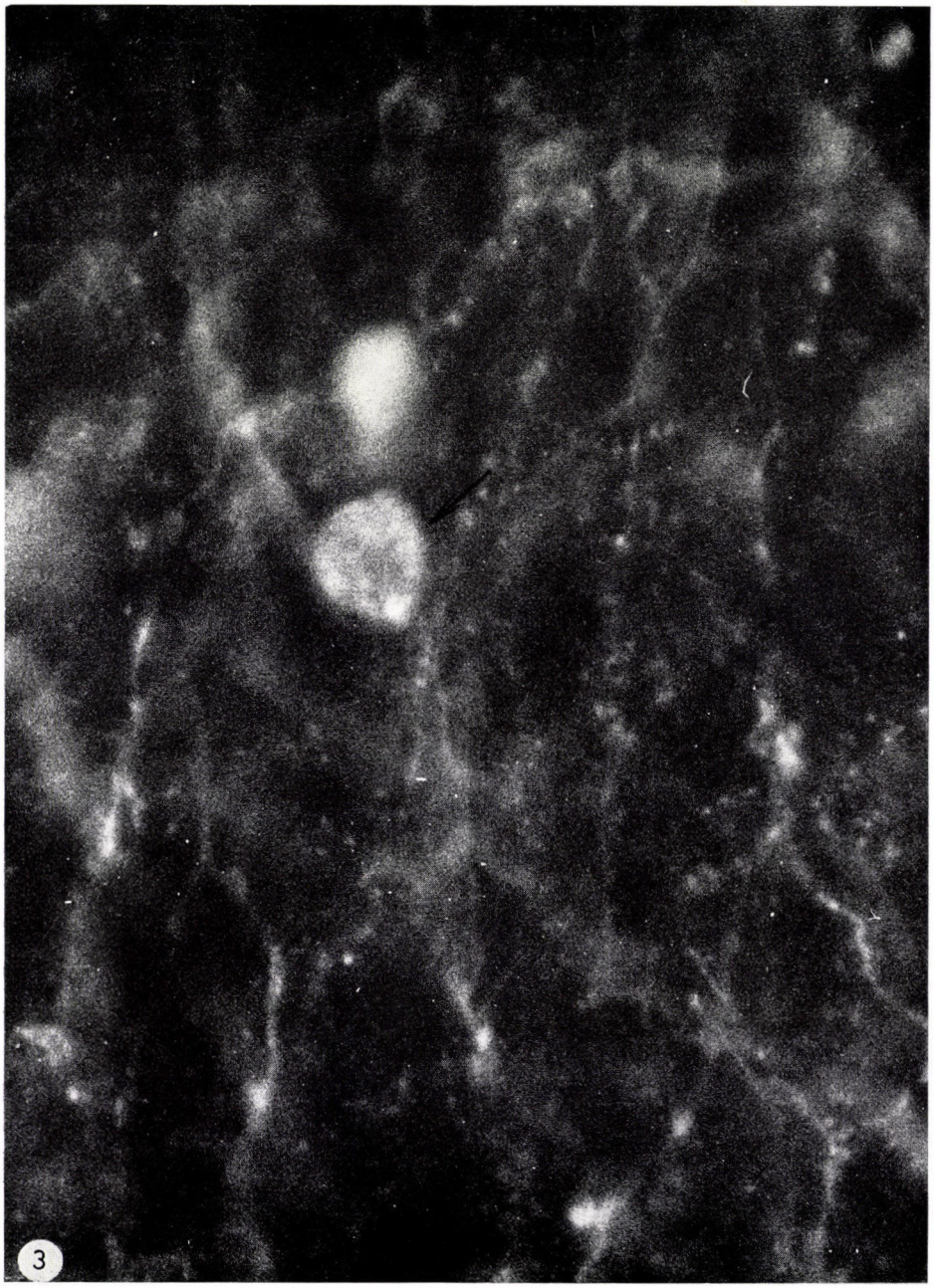

Fig. 3. Fluorescence microscopic pattern of "spherical bodies" in the course of normal development of the adrenergic ground plexus of the rat iris. 10th postnatal day. Axons exhibit a green fluorescence, whereas fluorescence colour of spherical body (arrow) is yellow; × 2 000

Table 1

Specific fluorescence of spherical bodies; in vivo and in vitro experiments performed on irides of 6-day-old rats

Substance (drug)	Dose (concentration)	Time	Results	
			Fluorescence	Toluidine blue staining
Reserpine, *in vivo*	5 mg/kg	24 h	normal	
Reserpine, *in vivo*	3×5 mg/kg	each 6 h	decreased	
α-methyl-m-tyrosine, *in vivo*	400 mg/kg	12—24 h	normal	
Serotonin, *in vivo*	100 mg/kg	2— 6 h	increased	
C. 48/80, *in vivo*	5 mg/kg	12—24 h	minimal residual	degranulation
C. 48/80, *in vitro*	100 μg/ml	1— 5 min	minimal residual	degranulation
Polymyxin B, *in vivo*	5 mg/kg	12—24 h	minimal residual	degranulation
Polymyxin B, *in vitro*	100 μg/ml	1— 5 min	minimal residual	degranulation

to an early decentralization, the number of mitotic figures of such cells is strikingly high. In early stages of mitosis, these cells do not contain any metachromatic granules (Fig. 4a); thus, it appears that they originate from a non-differentiated cell type. However, as soon as the process of mitosis approaches completion, metachromatic granules appear within the cytoplasms of the dividing cells (Fig. 4b). These fill up the cytoplasms of young mastocytes (Fig. 4c). Even in mature mastocytes, however, the metachromatic granules vary considerably in number (Fig. 4d).

The metachromatic mastocytes were counted in several control and decentralized irides. Their number was 182 ± 15 in controls, whereas 474 ± 31 in irides subjected to decentralization during the first week of postnatal development. This 2.5-fold increase is highly significant.

Discussion

According to literary data post- and preganglionic denervation (decentralization) induce fundamentally different alterations in the adrenergic transmitter content of autonomically innervated tissues of adult animals.

Thus, biochemical studies [1—3, 7, 10, 12, 20] and histochemical investigations [8, 9, 13—16] unequivocally have shown that catecholamine contents of adrenergic terminals are depleted after denervation, due to deterioration of the transmitter storage mechanism.

On the other hand, no changes in the catecholamine content of adrenergically innervated tissues were ever reported after decentralization performed in adult animals. Thus, while studying the noradrenaline levels in the cat nicti-

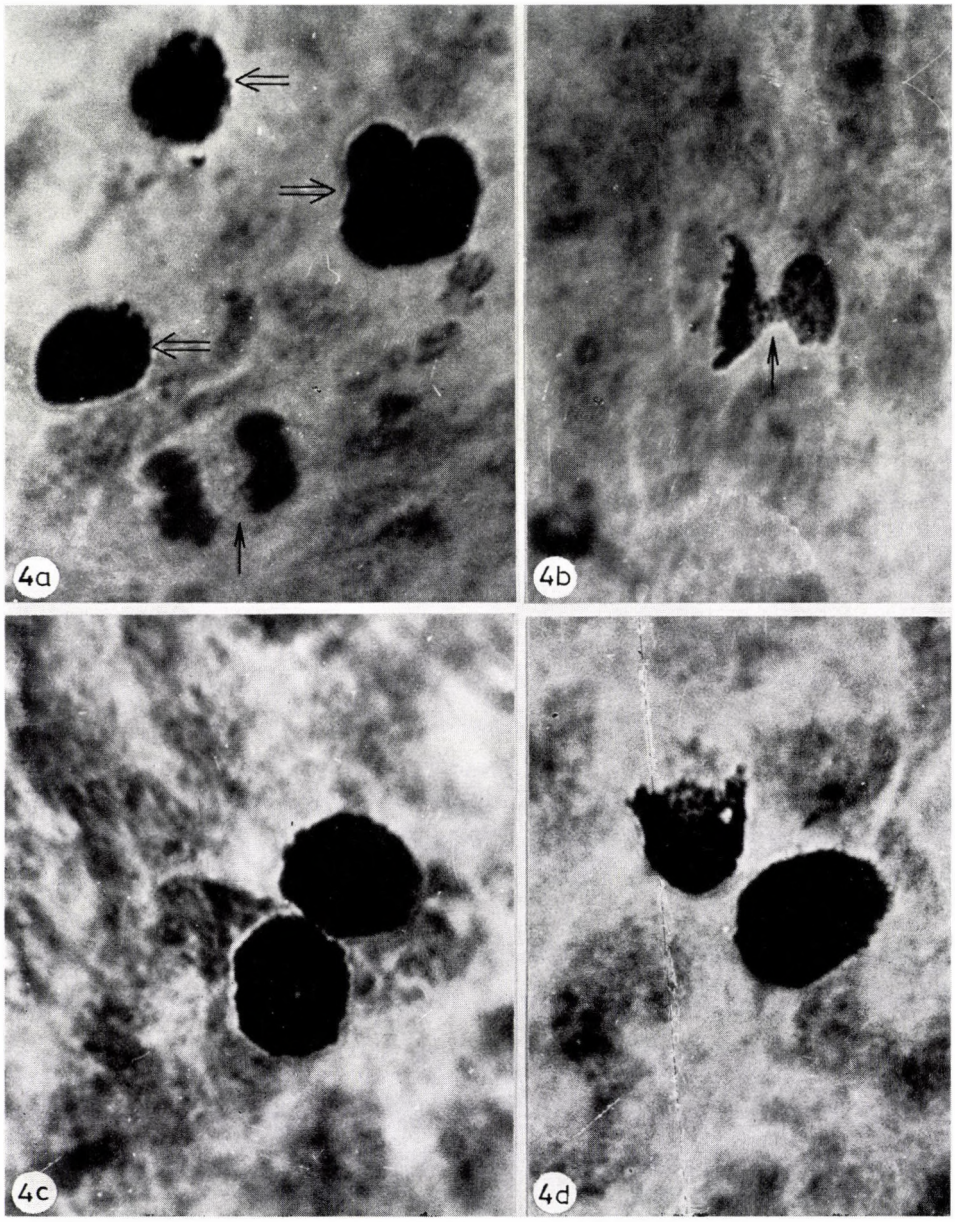

Fig. 4. Mastocytes containing metachromatic granules in the iris of a 9-day-old rat subjected to preganglionic denervation (decentralization) on the 2nd postnatal day. Toluidine blue staining. × 2 000. a = mitotic cell, devoid of metachromatic granules (arrow), mature mastocytes (double arrows); b = first appearance of metachromatic granules (arrow) in one of the daughter cells deriving from a mitotic mastocyte. Note the cytoplasmic bridging between the two daughter cells; c and d = pairs of young mastocytes, loaded with metachromatic granules

tating membrane after transection of the preganglionic fibres of the ganglion cervicale superius, KIRPEKAR and co-workers [12] did not find any alterations; similar results were obtained by REHN [17] in the cat spleen and kidney after transection of the splanchnic nerves. Also in histochemical experiments, FALCK [8] obtained noradrenaline-fluorescence pictures identical with those in the controls in the rat iris after preganglionic denervation. Recently, EDVINSSON and co-workers [6] have found an unchanged fluorescence activity of decentralized sympathetic nerve fibres in rabbit iris and choroid plexus with a decreased total noradrenaline amount, however. At the same time, both fluorescence intensity and noradrenaline content increased in (or rather around) pial blood vessels. Thus it seems possible that different neurones possess different transmitter turnover. This is in accordance with SEDVALL's [18] notion that noradrenaline levels of postganglionic sympathetic neurons subjected to deafferentation (i.e., decentralization) are dependent on the decreased physiological activity and the relatively decreased transmitter synthesis, representing a special counteracting mechanism.

The present investigations suggest that similar mechanisms may influence the development of the adrenergic ground plexus in early stages of ontogenesis. We found, namely, that a decentralization performed during the first postnatal week, results in a conspicuous retardation of the formation of postganglionic structures. On the other hand, we found a luxurious hyperproliferation of immature postganglionic structures after decentralization performed in the second postnatal week. Thus, it may be assumed that, in this "critical period" of development of autonomic ganglion cells, central connexions of these cells exert some kind of a regulative (more properly: restrictive) effect, ensuring structural maturation of the ground plexus and impeding hyperproliferation of nerve fibres. Decentralization performed in this "critical period" appears to cut off this regulatory effect; accordingly, the ground plexus remains retarded in its development or, if performed in a later stage, it is "released" from the regulation, resulting in an imperfect hyperinnervation. This "critical period" seems to coincide with the last moments of functional inactivity of the rat iris, being functionally activated only on the 11th—12th postnatal days, after opening of the eyelids. This assumption is consistent with JACOBSON's [11] observations, suggesting that the "last maturation", i.e., functional activation of neurons is initiated by a specific trans-synaptic excitation. It appears, furthermore, that after this period, activation of these neurones impedes further activation of imperfect synapses, produced by axonal proliferation.

Also the important fact that, after an early decentralization, the normally characteristic involution of "spherical bodies" characteristic of the normal course of development, does not take place, merits consideration. Since these spherical bodies (or, more properly, tissue mastocytes containing these

bodies) show multiplication after decentralization, it may be suggested that these special mastocytes, observed already by DE CHAMPLAIN and co-workers [4], are in a causal relationship with the structure, function and genesis of the autonomic ground plexus. Recently, also STACH [19] has noted the consistent participation of mastocytes in the structural organization of the autonomic ground plexus. It needs further studies to disclose whether the increased number of mastocytes has anything to do with neurotransmitter processes, or rather with permeability factors, involved in the genesis and function of the peripheral autonomic innervation apparatus.

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Acta biol. Acad. Sci. hung., **24**(3—4), 233—235 (1973)

HYPOPLOIDY IN BRAIN TUMOURS

SHORT COMMUNICATION

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(Received 1973—06—25)

Occurrence in brain tumours of chromosomes below the diploid number has been reported [6], the lowest diploid count has been a stem line of 38 chromosomes [6, 7]. COX and co-workers [1] reported a widely varying number of double minute chromatin bodies with no chromosomal breakage in three neuroblastomas, one medulloblastoma and one rhabdomyosarcoma. Since then numerous workers [3—5] have confirmed their occurrence in tumour cells; both in hypoploid as well as hyperploid counts. We record here under the diploid number of 26 chromosomes in medulloblastoma, ependymoma and anaplastic glioma. Further, medulloblastoma tissue has exhibited [2] extreme hypoploidy by showing merely a dozen swollen chromosomes. Similarly, new cell lines with conspicuous gross chromosomal rearrangements have been found, exclusively in malignant cell populations. Therefore, the finding of a chromosomally rearranged cell line or other chromosomal aberrations in ependymoma and tuberculoma would be an entirely new discovery of tremendous significance.

DHARKER and co-workers [2] have described the methods of investigation. Briefly, immediately on removal, tumour tissue was divided into four parts, (a) first part was fixed in Newcomer's fixative; (b) second, placed in saturated aqueous paradichlorobenzene for 2½—3 h for pre-treatment; (c) third, in 1% glucose for 2 h; and (d) fourth, sent to the Department of Pathology, G. R. Medical College for histopathological analysis. Tissue placed as (b) was transferred to fixative and (c) was then transferred to paradichlorobenzene for 3 h and finally fixed in the fixative.

Out of 14 brain tumours studied the above-mentioned schedule could be followed only for eight tumours. Best aceto-orcein stained squashes were obtained from the tissue processed as (c). However, an entire schedule could not be followed for every tumour.

Data on metaphase counts are shown in Table 1 which informs that hypoploidy is equally common in malignant as well as in benign tumours.

Especially medulloblastoma is characterized by an extreme degree of hypoploidy; nearly 70% metaphases revealed hypoploid counts (Fig. 1) which in-

cluded 20% cells showing only 9—12 swollen chromosomes. In agreement with previous workers [1, 3—7] small chromatin bodies were often observed but not more than 3 or 4. Breaks showing tightly paired chromatids without centromere were also seen. Chromosomes in ependymoma, despite its benign nature, were frequently observed; this was also true for astrocytoma and

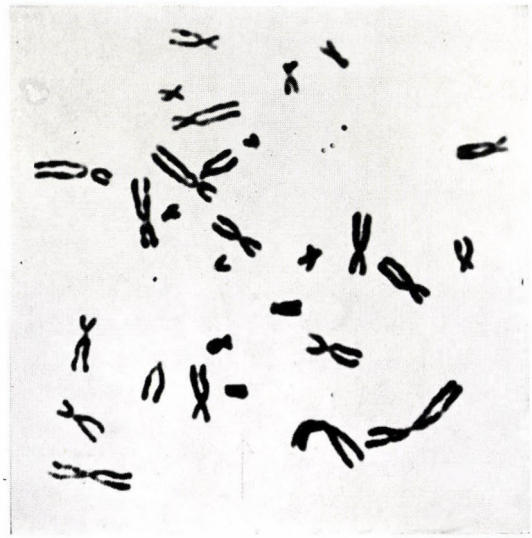

Fig. 1. 2n = 28 chromosomes of medulloblastoma obtained after recovery in glucose. Note chromatin droplets and a tightly paired chromatid of an acentric fragment; × 1 250

tuberculoma. The lowest count in all tumours except medulloblastoma [2] has been 28 ± 2. Even this is highly variable and unexpected, for MARK and others [3, 6—7] recorded 36—38.

Table 1

Metaphase analysis of brain tumours

Tumour type	No. of cases studied	No. of metaphases	Cells showing counts			
			Hypoploid		Hyperploid	
			N.	f.	N.	f.
Malignant						
Anaplastic glioma	1	24	16	0.666	8	0.334
Medulloblastoma	2	82	59	0.718	23	0.282
Benign						
Astrocytoma	1	14	10	0.714	4	0.286
Ependymoma	2	74	66	0.891	8	0.109
Tuberculoma	2	19	12	0.631	7	0.369

While we have encountered several metaphases in each kind of tumour, we have not found all chromosomes uniformly spread, such as in the figure. It cannot be said therefore which group of chromosomes is subjected to loss or gain during variable ploidy grades of the respective brain tumours.

Nevertheless, data indicate that mosaicism in chromosome number cannot be the monopoly of pathogenesis of malignant tumours.

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Acta biol. Acad. Sci. hung., **24**(3—4), 237—239 (1973)

PREVENTION OF INDUCED AUTOPHAGOCYTOSIS BY THE PROTEIN-SYNTHESIS INHIBITOR EMETINE

SHORT COMMUNICATION

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(Received 1973—09—21)

In recent years several data have been published concerning the effect on cellular autophagy of translational inhibitors of protein biosynthesis. Some of these inhibitors, like puromycin [4] and dimethylnitrosamine [2], were reported to induce autophagocytosis in the cytoplasm of the damaged cells. On the other hand, the glutarimide antibiotic cycloheximide caused no considerable fine-structural alterations in the cytoplasm and it was even found to be capable for preventing autophagocytosis induced *in vivo* by either of neutral red [6], puromycin [4], a hyperosmotic solution of sucrose and cadmium ions [7].

In the present work, the effect of the ipecac alkaloid emetine, a ribosomal inhibitor of peptide bond formation [1] was tested on autophagocytosis induced by neutral red in mouse pancreatic acinar cells *in vivo*.

Mice were given neutral red 0.4 mg/g body weight intraperitoneally. As expected, a massive autophagocytosis was seen in the acinar cells 3 h later (Fig. 1). If mice were given a single non-lethal dose (either 0.01 or 0.005 mg/g b.w.) of emetine 30 minutes prior to neutral red, no autophagic vacuoles were observed in the cells 3 h after the injection of the dye (Fig. 2). When neutral red was administered 24 h after the same doses of emetine, autophagic vacuoles did appear. Emetine alone did not cause any detectable fine-structural alterations in the cytoplasm as compared to the untreated controls. According to our preliminary results, emetine is capable of preventing neutral-red-induced autophagy also in the epithelial cells of the seminal vesicle.

In accordance with previous data, the present results have shown that, based on their effect on cellular autophagy, translational inhibitors can be classified into at least two groups. Some of them, like puromycin, ethionine and dimethylnitrosamine, *induce* autophagocytosis, while others, including cycloheximide and emetine, *are protective* against induction. Since all of them inhibit protein biosynthesis at the ribosomal level, the differences in their mechanisms of action should be taken into consideration when trying to explain their divergent action on autophagy. A breakdown of polyribosomes followed by degranulation of the rough-surfaced endoplasmic reticulum is

characteristic of the actions of the inhibitors causing autophagocytosis as well [see Refs 6 and 8]. Contrary to this, cycloheximide inhibits peptide bond formation mainly through inhibition of translocation, and thereby freezes polyribosome structure [5]. The mode of action by which emetine inhibits peptide bond formation is similar to [1], though not identical with [3], that of cycloheximide. Emetine inhibits the aminoacyl-tRNA transfer reaction in protein

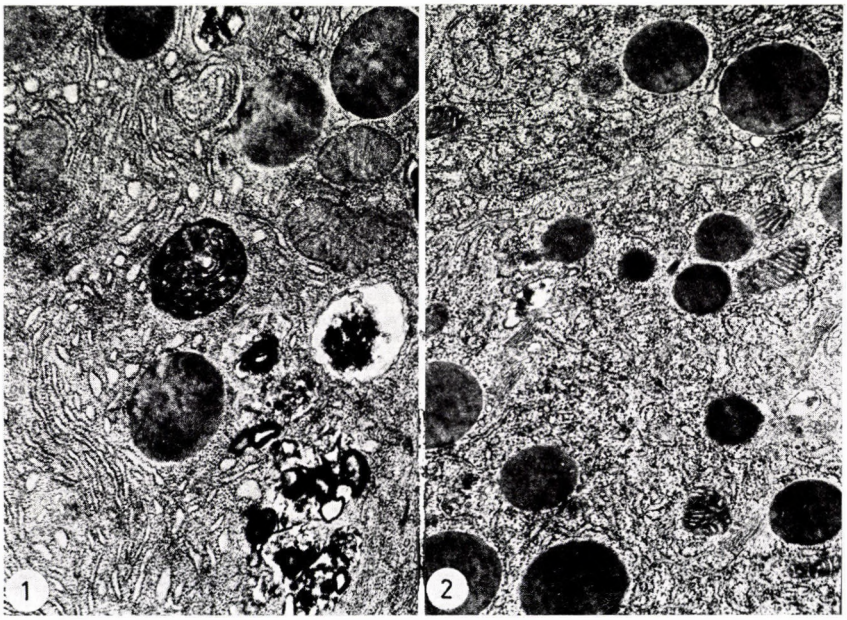

Fig. 1. Part of acinar cell 3 h after the treatment with neutral red. A number of autophagic vacuoles are present in the cytoplasm; × 12 000

Fig. 2. Pancreatic cell 3 h after the neutral red treatment from mouse pretreated with emetine. No dye-induced autophagic vacuoles; × 10 000

biosynthesis. If it is added after protein synthesis has been initiated, the nascent peptides remain firmly attached to the freezed polyribosome structure [1]. Therefore, the fact that pretreatment with emetine is protective against induced autophagy, is consistent with our previous hypothesis, that the stabilization of polyribosomes may be followed by a preservation of the rough-surfaced endoplasmic reticulum. This would mean an inhibition of a membrane transformation considered to be necessary for the formation of the limiting membranes of the autophagic vacuoles [6, 7].

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RECENSIONES

FRAZER, J. F. D.: **Amphibians**
The Wykeham Sciences Series 25. Wykeham Publications, London (1973), pp. 122, £ 2. 0.

This book gives a brief survey of amphibians, a class of Vertebrata, and discusses some interesting details of their lives and the unsolved problems of their way of living.

In addition to the preface, the book contains 12 independent chapters followed by two appendices and a subject and author index. The first 5 chapters summarize the systematics and evolution, the geographical range and speciation, the anatomy and physiology, the ecology and behaviour, and the larva (tadpole) of amphibians. Four chapters describe the way of living of Amphibia in different habitats. Within the latter problem, special chapters are devoted to the humid environment, the adaptation to arid environment, representatives living above soil level, and to adaptation to exclusively aquatic habitat. The remaining 3 chapters summarize amphibian biocenoses, the relations of amphibians to man, and the problems and techniques of investigations. The chapters each form self-reliant units, viz. reading one chapter does not suppose the knowledge of the others. The first of the appendices lists periodicals and some general and local publications on amphibian research while the second one presents the system of amphibians broken down to the genus. The book is ended by an index promoting orientation and it is illustrated by a good many photographs, maps, and figures.

The book may be considered as a popular work of high level. When writing the book, the author has had the intention to familiarize those interested in nature with the life of amphibians, on the one hand, and to call the attention of the young zoologist generation to the research on the life of this group of animals, on the other hand.

O. G. DELY (Budapest)

PORTER, K. R.: **Herpetology**
W. B. Saunders Co., Philadelphia—London—Toronto (1972), pp. 524.

This handbook summarizes our basic knowledge on herpetology. In addition to the preface, it is divided into 15 chapters. Chapter 1 is an introduction which offers a brief survey on the history of, the prominent scientists, primarily those in America, engaged in, and on the modern trends of, herpetology. The introduction then familiarizes the reader with the societies functioning and the periodicals publishing in this branch of science. The other chapters discuss amphibians and reptiles belonging to the subject, using individuals, active members of whole populations and biocenoses, to illustrate the structure and function of their organism. Thus, special chapters are devoted to the morphological and functional characteristics, the origin and the phylogenetic relations of the different groups, their geographic range, the role of water and moist required for their normal life functions and for their interaction with the environment, their colour and adaptive colour changes, the problems of their nutrition, biology of reproduction, the reproductive isolation of the species, the population dynamics of the more important groups, and finally, their relation to man. The book is concluded by a list of the scientific terms and a subject and an author index.

The author primarily discusses the to-day-living (recent) animals and uses paleontologic material in so far as it is necessary to elucidate anatomical and biological relations. He refrains to get entangled in the problems of systematics, but simply follows the system developed thus far. Whenever it is possible, the material of the chapters each is presented in context rather than isolated. The text is exquisitely completed by photographs, maps, tables, figures, etc.

This book is very useful for all those who wish to get engaged in herpetology and who are interested in amphibians and reptiles.

O. G. DELY (Budapest)

HORTOBÁGYI, T.: **The microflora in the settling and subsoil water-enriching basins of the Budapest Waterworks**
Akadémiai Kiadó, Budapest (1973), pp. 340, Figs 610.

This work concerns some practical questions. The first half of the book points to the problems arising during the everyday function of Budapest Waterworks. In order to solve the problems, a team has been organized, under the leadership of Dr. Á. BERCZIK, for carrying out the scientific evaluation of general hydrobiological, ecological and cenological investigations on the settling and subsoil water-enriching basins as well as the filtering systems. The author of the book under review investigated the microflora.

Synchronously parallel series of samples were taken in order to obtain appreciable complex data (physical, chemical, botanical and zoological ones). The samplings were carried out between May 1968 and October 1969, altogether 42 samples were taken. The author determined 415 taxons and classified them into 116 genuses. Of them 238 taxons proved to be new for the flora of the river Danube, and 58 of them have been new for the science at all. Among the algal phyla green algae showed the highest frequency, 307 species of them were encountered, 266 of them could be classified into the order of *Chlorococcales*, 129 of these belonged to the genus *Scenedesmus*. Phylum *Chrysophyta* was represented by 47 species, 39 of them were Bacillariophyceae. Phylum *Cyanophyta* was present with 33 taxons. Algal bloom was induced by the mass occurrence of a *Microcystis* species. It has been established during the investigations carried out at home and abroad that the frequent occurrence of *Planctomyces békefii* GIM. in flowing and standing waters indicates an increasing pollution. The plankton of the Danube being rich in organisms during summer contains 17—31 millions of algal individuals per liter. According to the parameters of the known saprobiological systems, the river Danube belongs to the beta-mesosaprobic ones and so does the settling basin, whereas the enriching one, just as a consequence of the algal bloom, can be considered as a beta-alpha-mesosaprobic one. Author follows the list of eurytherm and stenotherm species, compares the seasonal data obtained in the water of the Danube, the settling and enriching basins. The detailed description of the species being new for the science as well as the sizes and frequency of occurrence of all the species investigated and their place in the saprobiological system are to be found on 62 pages. The phytocenological data of the 42 samples including the places and dates of samplings, the water temperatures, the percentage of the individual numbers of predominant species as well as the percentual distribution of the algal phyla are described on 13 pages.

The results of the valuable limnological work being of significance even from points of view of technical practice are summarized in 21 points. The list of references covers 5 pages containing the works of numerous home and foreign scientists.

The book is illustrated by 610 artistic wash-drawings covering 138 pages. The details of the investigations, the documentation and summary are of highest value for the limnologists and even for the technical practice.

GIZELLA TAMÁS (Tihany, Hungary)

Advances in molecular genetics
Ed. W. HAYES, British Medical Bulletin, Vol. 29 (3), Medical Department. The British Council, London (1973)

One can only envy the editor for his possibility of finding excellent experts in the vast field of modern molecular genetics in a single country and also for his — certainly well-established — belief that the book will be read by physicians.

To learn molecular genetics seems to be inevitable for every biologist including physicians in medical research, but one wonders if this necessity matches with the actual ability of the medical researchers to digest all that is so well condensed in these papers.

This number of the Bulletin summarizes our current ideas in the framework of molecular biology about how the living systems operate. There are fifteen papers with the following titles: DNA synthesis; Replication of DNA in eukaryotic chromosomes; Molecular architecture of human chromosomes; Regulation of cell division in bacteria; Positive and negative control of transcription in bacteriophage λ; Control of transcription in bacteria; Genetic and structural analysis of transfer RNA; DNA repair and recombination; Molecular structure and function in RNA phages; Genetic studies on RNA tumour viruses and their hosts; Genetic analysis of animal viruses; Proteins of polyoma virus and SV40; Nuclear transplantation and regulation of cell processes; Cytoplasmic genetic systems of eukaryotic cells; The genetics of behaviour.

Only five of the 15 papers are concerned with viruses and bacteriophages but even the number of papers dealing with eukaryotic cells is not more than five. There does not seem a breakthrough as yet in applying molecular genetics in understanding the more complex living systems.

G. Szabó (Debrecen)

Szórády, I.: **Pharmacogenetics. Principles and pediatric aspects**
Akadémiai Kiadó, Budapest (1973), pp. 240.

One of the most important problems worrying the population of the world is the question of pollution and vitation of the environment. Thousands of compounds are prepared and introduced every year. We meet these substances in several ways, either by eating, drinking, breathing and wearing them, or by using them as drugs. The utilization of the new compounds is highly beneficial for mankind, as they usually make life easier and support our fight against several diseases. Nevertheless, some of these substances in some individuals may cause unexpected, deleterious effects depending on the genetic constitution of a person receiving a drug.

A comparatively new branch of the genetic sciences is dealing with the study of genetically determined variations in animals and humans which are revealed by the effects of drugs. This new branch is pharmacogenetics. About 10 years ago just a few examples were known (like acatalasia, primaquine sensitivity, suxamethonium sensitivity, slow and rapid inactivation of isoniazid, PTC taste-testing polymorphism, and G—6—PD deficiency), while nowadays, hundreds of enzymes or enzyme systems have been recognized in the human organism, all of them being genetically determined, thus having the possibility of missing or alteration.

In spite of the superabundant mass of information in this field, there are only very few books dealing with pharmacogenetics, as it can be seen in the key references of Dr. Szórády's book, where only three basically pharmacogenetic works could be mentioned (Kalow's, Lőhr and Waller's and Meier's books). This comes from the fact that pharmacogenetics can be considered as pharmacology by the geneticists and as genetics by the pharmacologists. From this point of view Dr. Szórády's book is supplying a great want, and is of great importance.

Dr. Szórády is a pediatrician with a deep interest in clinical pharmacology, and it is easy to understand that he is discussing pharmacogenetics as a part of clinical pharmacology, as it can be read in Chapter I, but as soon as he is summarizing pharmacogenetic enzymopathies in Chapter II, he becomes a true geneticist. Among the clinical aspects of pharmacogenetics (Chapter III) the main attention is paid to pediatrics, but other branches of medicine (internal medicine, neuropsychiatry, obstetrics and gynecology, surgery and anaesthesiology, dermatology and allergology, etc.) are also dealt with. Thus the book which is completed by three additional chapters on experimental pharmacogenetics (Chapter IV), pharmacogenetics in preventive and social medicine (Chapter V) and the teaching of pharmacogenetics (Chapter VI), gives full satisfaction to pharmacologists, geneticians and clinicians as well.

The best pages of the book are those dealing with the biochemical and clinical consequences of the temporarily immature, insufficient enzyme set of the premature or even of the mature newborn babies, which are (as Dr. Szórády was the first to point out) similar to those caused by real, genetically determined enzyme defects.

The reader who wants to deal more intensely with pharmacogenetics is strongly supported by the ample bibliography consisting of more than 1500 references.

The book, which is provided with a foreword by Professor G. Fanconi, can be recommended to advanced students in medicine and biology and to experts in biology and medicine who wish to get acquainted with the details, present state and future of pharmacogenetics.

The Publishing House of the Hungarian Academy of Sciences deserves credit for the realization of this book. Perhaps legibility could have been better helped by a little bit looser type-setting.

G. Szemere (Szeged)

Sleigh, M. A., MacDonald, A. G.: **The effect of pressure on organisms**
Cambridge University Press, Cambridge (1972), pp. 516, £ 8.0

The volume contains the lectures given at the 26th Symposium of the Society for Experimental Biology, concerned with the effects of pressure on living organisms. The first series of papers concerned the effects of pressure on physico-chemical features, on very excit-

ing facts on water structure, buffer systems and on the configuration of macromolecules; the second series of papers is devoted to the effects of pressure on enzyme systems and the integrated functioning of aquatic animals; the next papers discuss the gas-filled spaces, providing buoyancy and various other possibilities of maintaining it, their features related to pressure, the problems of compression and gas exchange during diving by air-breathing vertebrates, their special adaptation to long diving periods with collapse of the lung; lastly, problems of detection of pressure changes and their influence on animal behaviour are discussed. Among the reflexes concerned, the trigeminal nerve and, especially, the perinasal region play an important role.

The symposium comprehended a great variation of pressure, surroundings, temperature and biological species from the point of view of various disciplines, summarizing recent knowledge on influence of high pressure on biological processes and life in the Oceans. There are many references to human diving with important practical consequences. Facts and theory of nitrogen narcosis and its connection with the influence of pressure are discussed.

The volume is a highly interesting synthesis of knowledge won in the field of various sciences and opens new aspects of research in connection with change of environmental pressure and its influence on many processes in physics, chemistry, biochemistry and biology.

Instructive figures and plates help understanding the complexity of statements.

I. RÓZSAHEGYI (Budapest)

LIBBERT, E.: **Lehrbuch der Pflanzenphysiologie**
VEB G. Fischer, Jena (1973), pp. 472, M. 39.

Plant physiology closely follows the speedy development of biology and thus the excellent book written by the professor of plant physiology at the University of Rostock is warmly welcome. The book well reflects the present status of this area of science and discusses every modern problem of plant physiology, presenting 341 figures for illustration. The first three main chapters summarize the basic theorem bearing our present knowledge on metabolism and regulation from the general biologist's point of view. The following main chapters discuss explicitly the nutrition and internal transport of materials of plants, the problems of growth and development, and finally, the physiology of motion and excitation of plants. A special merit of the book is that it presents a clear and concise summary of the up-to-date material, making a scientific orientation possible for everybody interested. The book is rather instructive for both undergraduates and university instructors. The alphabetic index is so ample that it may be a good source of information for anybody interested.

V. FRENYÓ (Budapest)

Ultrastructural features of cells and tissues in culture
Symposia Biologica Hungarica, Ed. I. TÖRŐ and GY. RAPPAY. Vol. Akadémiai Kiadó, Budapest (1972).

This volume contains lectures presented at the Budapest Congress of the European Tissue Culture Society held in 1971. The 12 lectures were presented as main referates by the most famous representatives of tissue culture. The conferates and short papers presented at the Congress are not included in this volume.

I. TÖRŐ, the President of the Congress, dealt with the alterations in the fine structure of lymphoreticular cells. He investigated the relationship between the fine structure and enzyme activities of explantates of rat thymus after different periods of culture. While the epithelial cells of thymus appeared to have lost their fine structural elements indicative of specific functions *in vivo*, the submicroscopic structure of the fibroblasts remained unchanged. Probably, the cultured epithelial cells underwent functional changes, whereas the fibroblasts continued producing connective tissue fibres.

Dr. FRANKS investigated the fine structure of cell lines obtained from normal and tumour tissues. The former showed ultrastructural changes during culturing, independently of the animal species, i.e., the specific ultrastructural markers of the tissues disappeared. The cells resembled morphologically the endothelial and pericytic cells. In the author's opinion these cells may have originated from parenchymal cells of the tissues. On the contrary, the cell lines taken from tumour tissues preserved the characteristic fine structure of the original tissue.

TIXIER-VIDAL established that the fine structure of prolactin-synthesizing and elaborating cells of duck hypophysis was essentially the same in organ culture as in the intact animals. The fine structure of LH-producing cells of rat hypophysis in culture indicates a continuous synthesis. In her opinion, however, the hormone was decomposed by the abundant lysosomes. Addition of LH-releasing factor to the cultures resulted in an alteration of the cellular fine structure and an increase in the basic secretion of the LH cells. The ultrastructure of a cell line obtained from an estrogen-induced hypophyseal tumour indicated a very low basic secretion, confirmed even by bioassays.

A. KAHRI and his group studied the relationships between the fine structure and hormone production of embryonic rat adrenal gland. The ultrastructure of cells cultured without ACTH treatment was significantly different as compared to that known in intact animals. The hormone production of such cultures was also very low. After a 6-day treatment with ACTH, the fine structure of the cells regenerated and even the hormone production increased. Since the 21-hydroxylase and 11-beta-hydroxylase enzymes, playing a prominent role in the steroid synthesis, are to be found in the endoplasmic reticulum and the mitochondria, respectively, and the ACTH treatment altered first of all even the fine structure of those elements, the authors regard as proven the existence of a direct relationship between the morphological effect of ACTH on the cultured adrenal cells and the increased steroid synthesis.

The same problem was concerned in the next lecture. In cat, rat and human embryos no direct relationship was found between the fine structure and hormone production of adrenal cells. Even the poorly differentiated adrenal cells, showing an ultrastructure differing from that known in adult individuals, were able to produce a great amount of specific hormone after addition of ACTH. The authors assume that the steroidogenic and structural effects of ACTH may be segregated *in vitro*.

Dr. BUKULYA studied the fine structure of rat adrenal cells and their responsiveness during embryonic life. The differentiation of rat adrenal glands *in vivo* seemed to be influenced not only by the trophic effect of ACTH.

Small lymphocytes were the subject of extensive studies. BLAZSEK and his co-workers studied the morphological alterations of white blood cells as well as their survival in short culturing without mitogenic agents. They established that the granulocytes were the first to disintegrate, whereas small lymphocytes of unchanged structure were observed even in the 120th hour of cultivation. The numerous active macrophages appearing in the cultures and phagocytosing the destroyed cells, deserve special interest.

W. O. GROSS (Lausanne) described a method for embedding a single heart-muscle cell cultured in a monolayer for electron microscopy, allowing a light- and electron-microscopic identification of cell particles.

RÖHLICH and TÖRÖK reported on the fine structure of retinal pigment epithelium in organ cultures taken from chicken embryos of different ages. They observed a special effect of the T8 medium (TROWELL, 1959) on the ultrastructure of the cultured pigment epithelium.

H. E. STREET and his group described the fine structure of plant cells growing in suspension. They pointed out that the investigation of fine structure of living cells allows a more exact revelation of cellular functions. Numerous figures demonstrate the ultrastructure and its alterations during cell division of the maple cells growing and multiplying in suspension. The authors expect further information in cell metabolism from so-called steady-state cultures.

This volume, as the above examples indicate, contains useful information about the fine structure of cultured cells, maintaining or losing their specific functions, and offers a basis for discussions on the problem of relationships of cellular fine structure and specific functions.

ANGELA GYÉVAI (Budapest)

Grundlagen der Cytologie
Ed. G. Ch. HIRSCH, A. RUSKA and P. SITTE.
VEB Gustav Fischer, Jena (1973), pp. 790, Figs 605, 135 M.

From the point of view of cell-biologists it is important to review the ever increasing amount of electron microscopic and cytochemical results.

This very valuable book was written by many authors, all well-known specialists of their own subjects. For this reason the level of every chapter is high.

The introducing chapter gives a historical survey of the cytology and a short description of the cell structure. The second chapter summarized the most important and well- known techniques used in cytological researches. Two chapters give a molecular biological analysis of the cell-structure.

In the following chapters the nucleus of the interphase and mitotic cells, the structure and function of the centrosomes and cilia, and the other cytoplasmic organelles as well are described. Five chapters give a very good description of the different plant cells.

In the next main part of the book we can read about different animal cells. At first, the development and the structure of the sperm cell and ovum are discussed, then development of the skin of various species is demonstrated.

The epithelium of respiratory system, the haemopoietic cells as well as the different epithelial cells of the kidney are also described.

In the last chapter the most important biophysical, physiological and electron-microscopical data concerning the neurons, muscle cells and optical nerves are reviewed.

The text is completed with extraordinary good microphotographs and schematic drawings, praising not only the authors but also the careful work of the Gustav Fischer Verlag.

L. Kovács (Budapest)

The cell cycle in development and differentiation
British Society for Developmental Biology Symposium
Ed. M. Balls and F. S. Billett. Cambridge University Press, London (1973), pp. 483, £ 11, $ 32.50

The British Society for Developmental Biology held its 24th meeting at the University of Bristol in 1972. The papers delivered have been published in this book.

One of the most important field of the modern cell-biological researches is the observation of cell cycle. Cell cycle is studied by many authors from different aspects. For this reason it is not surprising that the "Cell cycle in development and differentiation" was the theme of the symposium.

Professor Mitchison, in his introducing paper, analysed the cell cycle from the aspect of morphogenesis and periodic gene expression. According to his thesis, both processes can be found in the cell cycle.

The exact determination of the length of the intermitotic period was the subject of Dr. Steels' paper. He summarized the different methods, and the technical problems were discussed.

Many papers were delivered on the determination of proliferative processes of different plants and invertebrates. These organisms seem to be good test objects.

Dr. Hamilton used early embryos of Amphibia to demonstrate how the different phases of cell cycle influence the radiosensitivity of proliferating cells.

The question of cell kinetics of foetal and adult erythropoiesis was dealt with in two papers, emphasizing the importance of erythropoietin.

Dr. Graham. studied the cell-cycle changes during the early phase of mammalian cell development.

Dr. Snow demonstrated the damaging effect of ^{3}H-thymidine on developing mouse embryo tissues.

The last paper, delivered by Dr. Rytömaa analysed the importance of the chalones in the control of mammalian cell division.

The publications of these works has been reasonable because the papers give a good information on the recent results of developmental biology.

The excellent presentation of the book praises the careful work of Cambridge University Press.

L. Kovács (Budapest)

Eiben, O. G.: **The physique of woman athletes**
The Hungarian Scientific Council for Physical Education, Budapest (1972), pp. 190, 94 tables, 55 figures

An outstanding group of characteristics of the polymorphism of the human race are the numerous parameters of the morphological constitution.

The individual variations and the individual differentiation are, of course, the resultants of several environmental factors in addition to the genetic background.

Data obtained by the methods of physical anthropology recognizing "man" from the human biological, viz., a unique point of view, offer an opportunity for a number of practical conclusions.

Modern sports, especially the achievements of the highest-level competitions and Olympic Game s, arouse interest in the physical properties of the sportsmen and sportswomen prominent in a branch of sport.

While a number of studies has already been performed on man athletes, the sport anthropology of woman athletes is an almost unexplored field.

This book presents the data obtained by the author on the most prominent woman athletes achieving topmost achievements in Europe. As controls, the undergraduates of the School of Physical Education and the Teachers' Training College and swordswomen were studied.

Body height, breadth, circumference and weight, and physiometric measures were taken and proportions were also determined. Expressing the results in relative measures, indices could also be formulated from which controlled data on the female morphological constitution could be obtained by appropriate mathematical apparatus. — Correlations of the body sizes were also calculated according to the principles suggested by TANNER. Total correlations were calculated for 9 subgroups of the woman athletes while partial correlations were calculated for several other groups.

In addition to the conventional anthropometric analysis, the method of analysis by the so-called generalized major axis has also been introduced by the author. (For details, the text of the book should be referred to.) The smart correlation vector analysis is apt for computation and programming. The values obtained appear in the system of co-ordinates as points and as clusters of points when related to more individuals, but at the same time they determine the characteristic location and area in the system of co-ordinates of the subgroup studied as consequent from the data of the measurements. Typical and characteristic overlappings can be found between the fields of the different athletical forms, while representation separates certain related forms of sport in the system of co-ordinates.

The book then offers a detailed analysis of the similarities, identities, and differences among, and constitutional characteristics of, the various kinds of sport.

Finally, the book raises the question: are women born as, or develop into, athletes? The answer is: they are both born as and develop into athletes (TANNER).

The value of the book is not only in the tremendous mass of data which, concerning woman athletes are completely new, but also in the introduction of the new method which is very useful for other physical and anthropological investigations in addition to the actual studies.

The book published in English is a promise to be an excellent book of reference.

GY. KISZELY (Szeged)

The physical maturity of nursery-school and school children in Budapest
Ed. HEGEDÜS, GY., EIBEN, O. Public Health Station, Budapest (1971).

The book is the work of a team of the following authors: Drs OTTÓ EIBEN, GYÖRGY HEGEDÜS, MÁTYÁS BÁNHEGYI, KLÁRA KISS, MARGIT MONDA, and ILONA TASNÁDI.

The editors' preface sets the purposes of the booklet: to observe and survey the children of nursery-schooling and schooling age for physical maturity in Budapest and to compare the results of the measurements performed in 1950 to those in 1960. The results of these measurements offer important data not only for pedagogues and physicians, but also for those who study the problems of urbanization and those of developmental acceleration in connection with the postponement of the upper limit of the schooling-age.

Since both the measurements and the evaluation of the data have been performed according to the instructions of the International Biological Program (IBP), the booklet may be considered a significant work of reference internationally as well. This characterizes the evaluability and the subsequent usability of the data. Cross-sectional growth studies, when performed on the ethnic group of an area, become interesting, if repeated, the role of environmental (natural and social) and population genetical factors can be judged.

In Chapter 1, the material studied and the methods used are described in detail. A total of 4 859 boys and 5 051 girls of the age between 3 and 18 years were studied in 27 kindergartens, 12 public primary schools, and 3 secondary schools during the school-year of 1968—69. The studies on children of the age between 7 and 14 may be considered as representative since public primary school is obligatory in Hungary. To make the sample representative, children in various districts of different characteristics of Budapest were studied. For the determination of age, the decimal system suggested by the IBP was used as described in the Appendix of

the book. The general points of view of the measurements, the instruments, the characteristics measured, the methods of evaluation, the calculation of indices as well as the biometrical methods are described in detail.

In Chapter 2, the results of the studies are presented and discussed. The body parameters (body height, sitting height, breadth across the shoulders, the breadth of the hip, the circumference of the chest, and body weight) are shown by tables presenting the necessary mathematical and biometrical values and then the way in which body measurements approach the values characteristic of adults is analysed. Relative body sizes and indices as well as normal zones are also presented. Finally, the physical maturity of the youth (3—18 years of age) of Budapest in the late 1960s is compared with the data obtained for the early 1950s.

The cross-sectional picture thus obtained may be correlated with economical and social changes that occurred during the period and have an indisputable practical and theoretical significance. The authors express their opinion that the so-called acceleration of development is possibly due to the cessation of the earlier retardation, though, the action of urbanizational and population-genetical factors is not negligible, either.

The interesting and instructive book reporting data of international interest, is closed by a complete list of the relevant Hungarian references and by a summary in English.

Gy. Kiszely (Szeged)

Spearman, R. I. C.: **The integument**
University Press, Cambridge (1973), pp. 208, £ 4.20

The integument is the subject of the third volume of the series published on the Biological Structure and Function. The author gives a comparative account of the integument in both invertebrates and vertebrates.

After a brief historical introduction, the book deals with methodological questions of morphological, biochemical and biophysical investigations necessary to study the skin. The first part presents the comparative morphology of the integuments of invertebrates and vertebrates, and devotes separate chapters to the integuments of lower invertebrates and *Coelomata*. One can also read about invertebrate skin colouration. Fish, *Amphibia*, reptiles, birds and mammals are dealt with in separate chapters. The second part gives an account of the comparative functions of the skin, the topics including thermal regulation, the decisive role of neuro-hormonal control, the synthesis of different components of the skin, the transport through the skin, immunology and development.

The book, a comparative account on the morphology and function of the integument, will be of interest to zoologists, dermatologists, pathologists and physiologists. Its excellent get-up and typography, the clear-cut drawings and high-quality microscopic pictures all bring credit to the editor.

L. Soltész (Budapest)

Scheuner, G., Hutschenreiter, J.: **Polarisationsmikroskopie in der Histophysik**
VEB Georg Thieme, Jena (1972), pp. 179, Figs 92, M 35,

The significance of polarization microscopy as an indirect method of ultrastructural research in biology has increased as a result of the growing interest in biological ultrastructure aroused by the direct visualizing method of electron microscopy. Unfortunately, at present only handbooks (mostly out of print) deal with the principles and application of polarization microscopy. This book intends to provide the basic knowledge of the theory of polarization optics in a short form with a useful description of the practical modern methods of polarization microscopy of histochemical significance.

The authors, a physicist (H.) and a biological morphologist (Sch.), have succeeded in giving a concise presentation of the theoretical principles of polarization optics and polarization microscopical methods with special emphasis on the quantitative aspects of histochemical selectivity.

In the first chapter the basic principles of polarization optics are described. The basic equations are introduced and many model figures are included, which are helpful in understanding the optical phenomena.

The second chapter deals with the different types of ultrastructural micellar patterns and their anisotropic effects, form and intrinsic birefringence, dispersion of birefringence, with a clear treatment of the phenomena of dichroism caused by an orderly arrangement

of selectively absorbing molecules in biological structures. The next chapter discusses the practical performance of polarization-optical investigations, the determination of the character of birefringence and the measurement of retardations with special respect to measuring the smallest retardations on biological structures. A chapter is devoted to polarization-optical histochemical methods. These methods are based on the specifically oriented association of colourless substances or dye molecules on the histochemically well-defined side groups of micellar textures or on the specific selective association of such components on micellar textures as a whole. The quantitative evaluation of these reactions is described and demonstrated with many graphs and tables.

This chapter will be of great practical value in showing, for the first time, the several possibilities of quantitative specific ultrastructural investigation on many biological structural components, as connective tissue ground substance, structural lipids, DNA and RNA, enzymes, neurosecretions and differentiation between scleroproteins.

The book fills a gap and therefore deserves to be welcomed.

G. Romhányi (Pécs, Hungary)

Hillman, H.: **Certainty and uncertainty in biochemical techniques**
Surrey University Press, Henly-on-Thames, Oxon (1972), pp. 126, £ 2.85.

The structure and the biochemical processes which underlie life cannot be studied directly in the enormous complexity of the living cell. Consequently, in the overwhelming majority of experiments life itself is first extinguished by either rigidly fixing cells and tissues or disintegrating them into their components. Only then can the increasingly sophisticated assays be carried out which, in turn, alter further the original characteristics of the sample. At the end of this long Odyssey we are compelled to face the crucial question: Are our data relevant to the understanding of life? or in other words: Do they faithfully reflect those properties of the living matter on which life is dependent?

Dr. Hillman tries to answer this basic question by carefully assessing the multiple effects on proteins of some widely used techniques, such as subcellular fractionation, histochemistry, electron microscopy, radioactive measurements, electrophoresis, and chromatography. With sharp criticism he analyzes each step of the procedures from the killing of the animal to that yielding the final result. It is the merit of the author that he successfully limits the discussion to the essential points without becoming lost in the evaluation of endless minor methodological variations. Some of his opinions are surprising and will certainly provoke fruitful debate. For example, he denies the mere existence of the endoplasmic reticulum, at least in the form visualized today. He argues that this three-dimensional network would restrict or entirely limit the movement of the three-orders-of-magnitude larger organelles easily seen under the light microscope. As this is not the case, the net ought to be stretched extensively, but the electron microscopic pictures do not show the so-expected, characteristic signs.

In spite of some inevitable exaggerations one has to share the final conclusion of the author: "A great deal of modern biochemistry of tissues *in vitro* is done with unknowing disregard of the laws of thermodynamics and physics. I would conclude that the validation of some of the most popular techniques in world-wide use is grossly overdue. Until and unless this is done, all the findings based on them must be regarded as unproven. If the necessary control experiments should fail to validate the techniques, the techniques should be abandoned. At the moment biochemistry is in the state of uncertainty because elementary control experiments for complex procedures have never been done. I submit that they should and must be done soon." Nevertheless, I feel strongly that the uncertainty is far from being as absolute as Dr. Hillman postulates. It should be remembered, for example, that *in vivo* experiments with radioactive tracers and those carried out *in vitro* on isolated organelles or enzymes are very often in essential harmony in spite of the unquestionable shortcomings of the methods used. Such corroborative results should reassure us that the present understanding of some biochemical processes is at least close to reality. On the other hand, the recent spectacular successes achieved in life sciences might undoubtedly induce false "security" feelings when applying these methods. The present book, therefore, should be read and discussed by all biologists, provided they seek remedy for this particularly dangerous ailment.

S. Kerpel-Fronius (Budapest)

International Symposium on HL—A Reagents
Proceedings of the 36th Symposium, organized by the International Association of Biological Standardization, June 1—3, 1972, Copenhagen
Ed. REGAMEY, R. H. and SPARCK, J. V., S. Karger, Basel, München, Paris, London, New-York, Sydney (1973), pp. 317, figs 65, SFr 60.

This Volume, No 18 of the Symposia Series on Immunobiological Standardization, presents the proceedings of the 36th Symposium held shortly before the 5th Histocompatibility Workshop at Evian in 1972. The aim of the Symposium was to review current developments in the standardization of transplantation antigens.

The book is divided into seven parts: (1) Production of HL—A antibodies; (2) Assay procedures and standardization of HL—A antisera; (3) Cellular interactions "*in vitro*" in relation to HL—A; (4) Serology of the HL—A system; (5) HL—A antigens, purification and chemical characterization; (6) Genetics of the HL—A system; (7) General biological function of transplantation antigens.

The first part discusses several methods for the production of HL—A antisera by planned immunization in human and heterologous relations. The lectures reported in the second part deal with the serological methods of HL—A typing and present some new methods for concentration and standardization of weak HL—A antisera. Here it was necessary to define the cellular interactions *in vitro* in relation to HL—A, i.e., to establish the correlation between HL—A typing and MLC response, and to study the cell-mediated lympholysis in man. The reports discussing the serology and genetics of the HL—A system were the most interesting parts of the Symposium. In order to improve the knowledge of the specificity of antigen-antibody reactions, the question was approached by the purification and chemical characterization of the transplantation antigens.

In the last part of the volume, some general biological functions of the transplantation antigens are considered from the clinical point of view.

This book is indispensable for immunologists and experts working in HL—A typing, and for surgeons being engaged in organ transplantation.

G. PETRÁNYI (Budapest)

Biocybernetics. Vol. 4
Proceedings of the IIIrd International Symposium on Biocybernetics. Satellite Symposium of the XXVth International Congress of Physiological Sciences, Leipzig, 3 to 7 August 1971.
Ed. H. DRISCHEL and P. DETTMAR. VEB Gustav Fischer Verlag, Jena (1972), pp. 320.

The book is a collection of the most interesting papers presented at the Symposium devoted to modelling biological systems and mathematical analysis of biological processes. Forty-six out of the about 80 presentations are included in this volume and arranged under the following five topics: (1) Basic, general and theoretical aspects of biocybernetics (11 papers). The papers deal among others with a pharmacological approach to modelling neuronal nets, the intracellular regulation of interneuronal connectivity — a basis of learning and memorizing processes, the basic processes of bioinforming activities in the brain. (2) Biocybernetics of the visual system (7 papers). (3) Biocybernetics of motor control (10 papers). (4) Biocybernetics of sensomotor systems especially eye and head pursuit movements (9 papers). (5) Biocybernetics of autonomous homeostatic systems, especially the cardiovascular system (9 papers). The summary of a round-table discussion on the main topic of the Symposium, held during the meeting (moderator W. D. KEIDEL), is also given in the book.

As pointed out by the Editors in the preface: "Papers, discussions and the round-table discussion have clearly shown that the first and by far too optimistic phase of cybernetic and systems approach in the field of biological research has been overcome". At the same time, the presentations have indicated that cybernetics is a very useful tool to gain new knowledge of life functions.

The selection of the lectures and the compilation of the material are excellent. The book is especially interesting to biocyberneticians and theoretical biologists, but all those working in various fields of biological sciences may benefit from it.

B. HALÁSZ (Budapest)

The Microflora in the Settling and Subsoil-Water Enriching Basins of the Budapest Waterworks

A comparative study in ecology, limnology and systematics

by T. Hortobágyi

Biospherical pollution and the eutrophization of natural waters are the problems of today. The author introduces in his present work the biocoenoses of the Budapest Waterworks. Detailed discussion is given on water production, on the physical and chemical condition of the basins, on the author's limnological and biological statements. In the taxonomical part, he deals with 415 taxa belonging to 116 genera beautifully illustrated in 610 original drawings. The author ascertained 238 taxa, new to the flora of River Danube; 58 taxa proved to be new to science. The work is concluded with the evaluation of the collectings; the inidividual phytocoenoses are compared in time and space.

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ULTRASTRUCTURAL FEATURES OF CELLS AND TISSUES IN CULTURE

edited by *I. Törő* and *Gy. Rappay*
(Symposia biologica Hungarica 14)

Tissue culture has become one of the basic methods of present day experimental biology. It is the purpose of the annual Conferences of the European Tissue Culture Society to promote the application of this method in the study of all kinds of cell-biological problems.

The papers in this volume are those of the first day of the 1971 meeting held in Budapest and all the contributions are dealing with the fine structure of cultured tissues and cells. The papers cover a wide range of experimental work with objects like lymphoreticular cells, tumour cells, endocrine as well as plant cells. The results indicate that irrespective of whether the original ultrastructure is retained or lost in culture, various functions characterizing the cells *in vivo* are still apparent under *in vitro* conditions. This symposium has been a further proof of the importance of the tissue culture method in studying the control of various vital functions on the cellular level. The electron micrographs presented are especially valuable to interested cytologists.

In English · 1972 · 159 pages · 96 photos · 7 figures · 16 tables · 17×25cm · Cloth

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PALEOGENE FOSSIL SPOROMORPHS OF THE BAKONY MOUNTAINS

Part I

by M. Kedves

(Studia biologica Academiae Scientiarum Hungaricae 12)

In his book "*Palynological Studies on Hungarian Early Tertiary Deposits*" (Akadémiai Kiadó, 1969) the author discussed the sporomorph composition of the Hungarian Early Tertiary deposits. The present work sets the target to elaborate monographically the sporomorphs of the Bakony Mountains. The complete work summarizing some 10 years of research will be published in four volumes in the series of Studia Biologica. The present, first volume gives the survey of the relevant literature on palynological investigation and the taxonomy of spores, together with the description of several new taxa.

In English · 1973 · 134 pages · 22 Tables · 12 Figures · Cloth

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A kiadásért felel az Akadémiai Kiadó igazgatója. Műszaki szerkesztő: Zacsik Annamária

A kézirat nyomdába érkezett: 1973. XII. 29. — Terjedelem: 11,2 (A/5) ív 55 ábra

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