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W dniach 22 i 23 września 1983 r. odbyło się w Krakowie pierwsze spotkanie biologów komórki. Pod egidą Polskiej Akademii Nauk i Uniwersytetu Jagiellońskiego zorganizowana została bowiem Pierwsza Ogólnopolska Konferencja Biologii Komórki. Głównym celem, jaki przyświecał organizatorom, a mianowicie Komitetowi Biologii Komórki, który jest powołany przy II Wydziale PAN, było stworzenie szerokiej platformy dyskusji dla polskich twórców biologii komórki, rozsianych po rozmaitych instytutach uczelnianych, Polskiej Akademii Nauk i resortowych. Zainteresowanie konferencją przeszło oczekiwania organizatorów. Napłynęło bowiem ponad 300 zgłoszeń. W efekcie na konferencję przyjechało 250 uczestników, którzy zgłosili 148 doniesień w formie plakatowej w 4 sekcjach. Organizatorzy konferencji podzielili obrady na 4 sekcje problemowe, które po swoich sesjach plakatowych obradowały w trybie dyskusji okrągłego stołu o charakterze plenarnym.

Sekcje problemowe obejmowały następujące zagadnienia: sekcja 1. struktury i funkcji błon biologicznych, sekcja 2. strukturalnych podstaw regulacji metabolizmu komórkowego, sekcja 3. ruchów komórek, sekcja 4. regulacji wzrostu i podziału komórek. Po wykładzie inaugurującym konferencję, a wygłoszonym przez profesora R. H. Lange * z Uniwersytetu Justusa Liebiga w Giessen (RFN) pt. Electron Microscopy of Periodic Macromolecular Arrays, nastąpiło wprowadzenie do obrad w sekcji wykładem, który wygłosił profesor Stanisław Przestalski, pt. Struktura i funkcja błon biologicznych. Następnie obradowały kolejno sekcje, których sesje były poprzedzane wykładami wprowadzającymi w problematykę danej sekcji i naświetlały obecny stan naszej wiedzy w zakresie problematyki sekcji. I tak w sekcji 2 wykład wprowadzający pt. Regulacja metabolizmu energetycznego i oddychania komórkowego wygłosił profesor Lech Wojtczak, w sekcji 3 profesor Leszek Kuźnicki scharakteryzował główne rysy ewolucji systemów ruchowych na poziomie komórkowym, zaś obrady sekcji 4 wykładem pt. Zależności cytoplazmatyczno-jądrowe w punktach kontrolnych cyklu komórkowego rozpoczęła profesor Maria Olszewska.

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* Prof. R. H. Lange zmarł nagle 8 sierpnia 1984 r.

Dyskusje okrągłego stołu prowadzili i koordynowali profesorowie: sekcji 1 Stanisław Przestalski, sekcji 2 Jerzy Kawiak, sekcji 3 Jan Zurzycki * i sekcji 4 Włodzimierz Korohoda. Po dwóch dniach obrad konferencję podsumował przewodniczący Komitetu Biologii Komórki profesor Jerzy Kawiak. Opublikowany w tym tomie zbiór nadesłanych streszczeń z prezentowanych doniesień jest pokłosiem Pierwszej Ogólnopolskiej Konferencji Biologii Komórki sponsorowanej wspólnie przez Polską Akademię Nauk i Uniwersytet Jagielloński.

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* Prof. J. Zurzycki zmarł nagle 27 lipca 1984 r.

POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3–4, 1984 (315–318)

OXYGEN UPTAKE SUPPORTED BY EXOGENOUS NAD(P)H IN AMOEBA MITOCHONDRIA

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Received: 23rd September 1983

Like mitochondria of plants [1] and of some Protista, the mitochondria of *Acanthamoeba castellanii* Neff oxidize the exogenous NADH in state 4 significantly faster than any substrate of the tricarboxylic acid cycle [2]. Moreover, in contrast to the respiration supported by those substrates the oxygen uptake due to NADH is highly inhibited by cyanide and only weakly stimulated by AMP, the activator of the alternative respiratory pathway [3]. The amoebae were cultured axenically with a constant agitation [4]. Mitochondria were isolated as in [5] from trophozoites from an early exponential phase of culture (generation time 8 hours, density 2×10^6 cells/ml). The oxygen uptake was measured amperometrically [5]. The degree of screening of the negative surface charge of mitochondria was measured with the fluorimetric probe 9-aminoacridine (9-AA) [6, 7].

In the state 4, NADPH is oxidized several times slower (optimum pH 6.2) than NADH (optimum pH 7.8). Uncoupling (+FCCP) shifts the optimal pH with NADPH from pH 6.2 to pH 7.0, while it does not do it with NADH (Fig. 1). The oxygen uptake supported by NAD(P)H is insensitive to rotenone and coupled with two phosphorylating sites. The stechiometry of O_2 consumed to NADH is 0.7; the $K_m^{NADH} = 20$ μM, $K_m^{NADPH} = 70$ μM.

State 4 oxygen uptake with NADPH (in presence of 0.2% BSA) is stimulated by 5–10 μM FCCP 2–5 times in various preparations. In absence of BSA already 1 μM FCCP is fully effective. Oxygen uptake in the uncoupled state decreases almost twice within 5 hours storage of the diluted preparation until 0.4% BSA is added, while the state 4 of respiration is unchanged. In the high-cationic incubation medium (120

mM KCl) the oxidation of NADH is twofold faster than in the low-cationic medium (0,25 M sucrose); in both media K_m^{NADH} is identical.

In state 4 EDTA (but not EGTA) strongly stimulates O_2 uptake, probably due to uncoupling following the removal of Mg^{2+} from the ma-

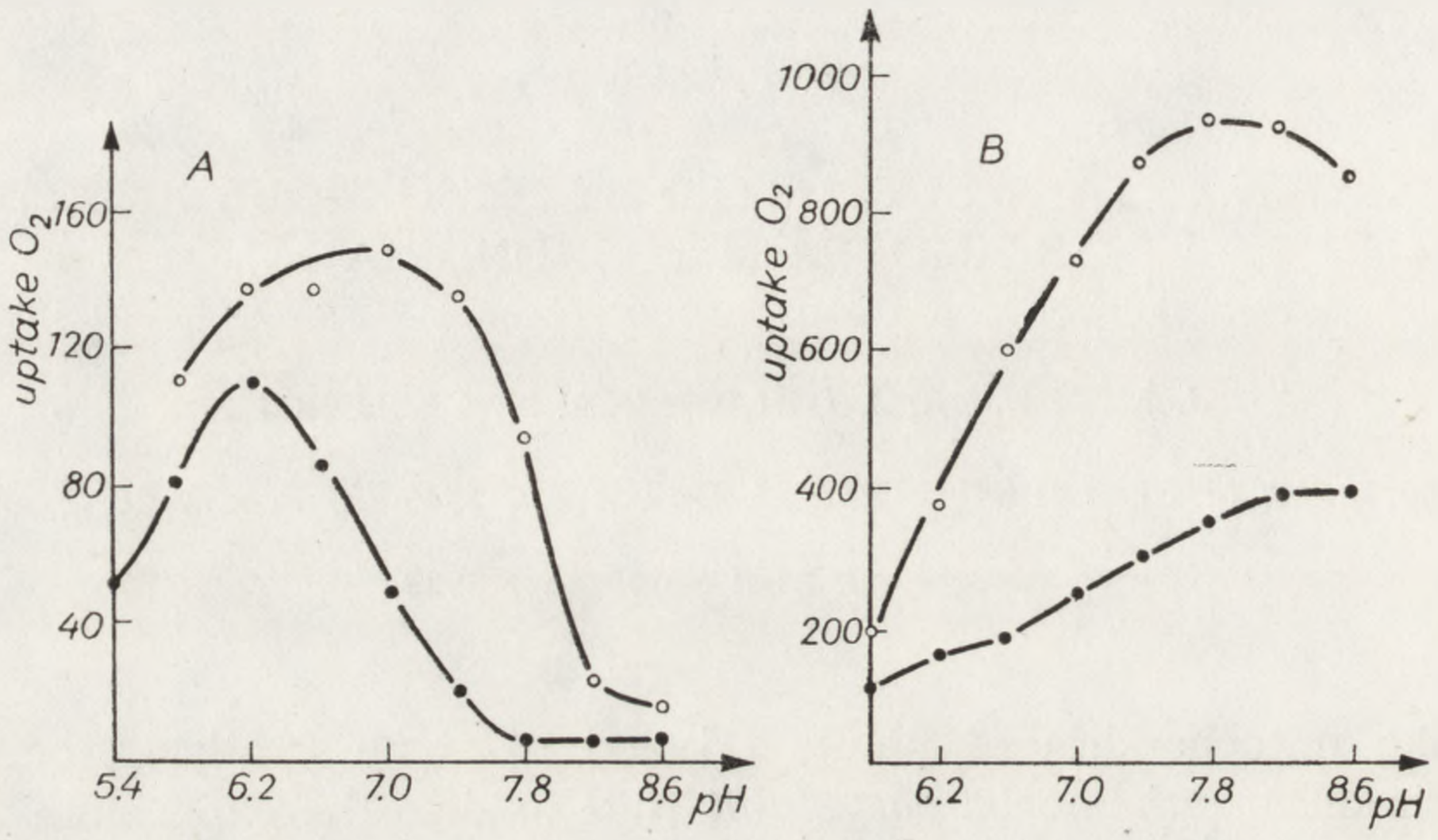

Fig. 1. Dependence of O_2 uptake supported by exogenous NADPH (*A*) and NADH (*B*) on pH

Incubation medium (1.5 ml, 25°C) contained: 120 mM HCl, 8 mM $MgCl_2$, 3 mM KH_2PO_4, 0.2% BSA, 30 mM MES, 30 mM HEPES, ∓ 5 μM FCCP; protein: 0.24 mg (st. 4), 0.12 mg (+ FCCP). O_2 uptake was initiated by the addition of 1 mM NAD(P)H

trix. In the presence of FCCP in a low-cationic medium both chelators decrease NADH oxidation by 20% in a manner reversed by Ca^{2+}, but

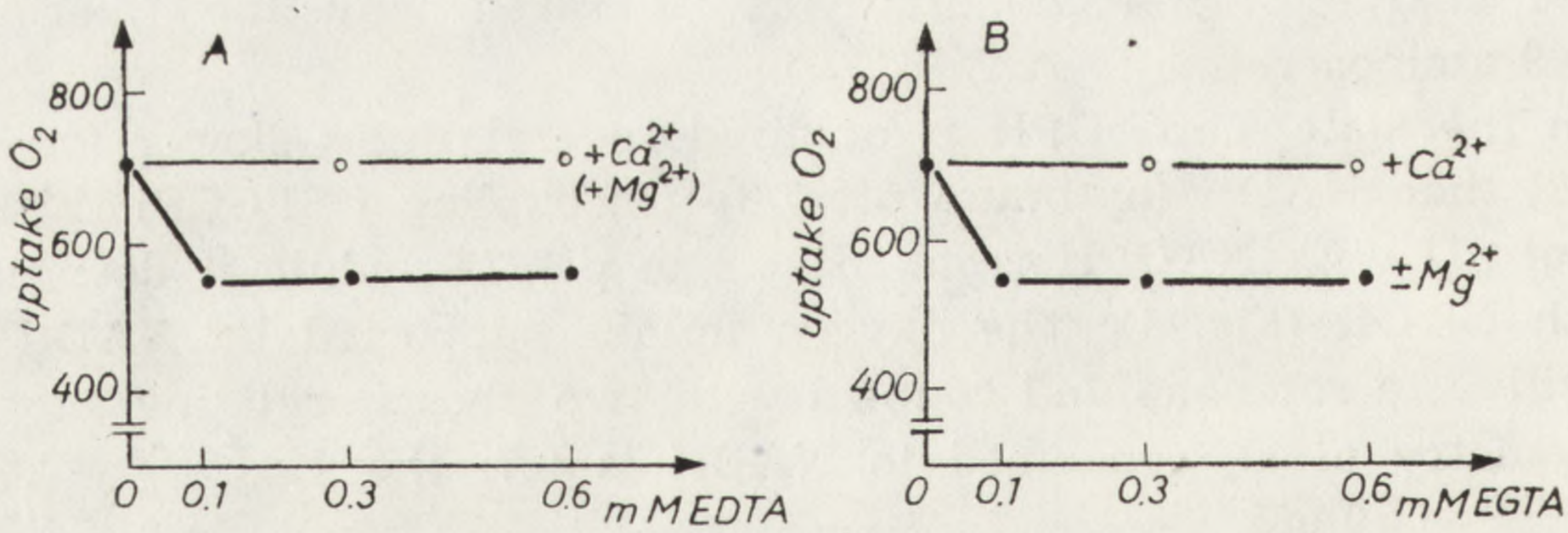

Fig. 2. The effect of chelators and of Ca^{2+} or Mg^{2+} on NADH oxidation; *A* — effect of EDTA (●) and of Ca^{2+} and Mg^{2+} (o), *B* — Effect of EGTA (●) and of Ca^{2+} and Mg^{2+} (o)

Incubation medium contained: 250 mM sucrose, 20 mM Tris/HCL pH 7.4, 1 mM FCCP, 0.3 mg mitochondrial protein; EDTA or EGTA at concentrations marked on the graph; oxygen uptake was initiated by 1 mM NADH. Ca^{2+} and Mg^{2+} were added during respiration

not by Mg^{2+}. The effect of Ca^{2+} does not seem to be due to the screening of negative charges, because in presence of a non-litic concentration of the cationic detergent CTAB [8], the inhibition of O_2 uptake by EGTA and its reversal by Ca^{2+} are the same as without CTAB. In absence of chelators neither Ca^{2+} nor Mg^{2+} have any effect on NADH oxidation. Measurements of 9-AA fluorescence revealed the presence in amoeba mitochondria of Mg^{2+} and Ca^{2+} in proportions depending on the conditions of mitochondria isolation: +EGTA = standard, +EGTA +Mg, + EDTA, without the chelators. In standard preparation three times more bound Mg^{2+} than Ca^{2+} was found, regardless of the presence

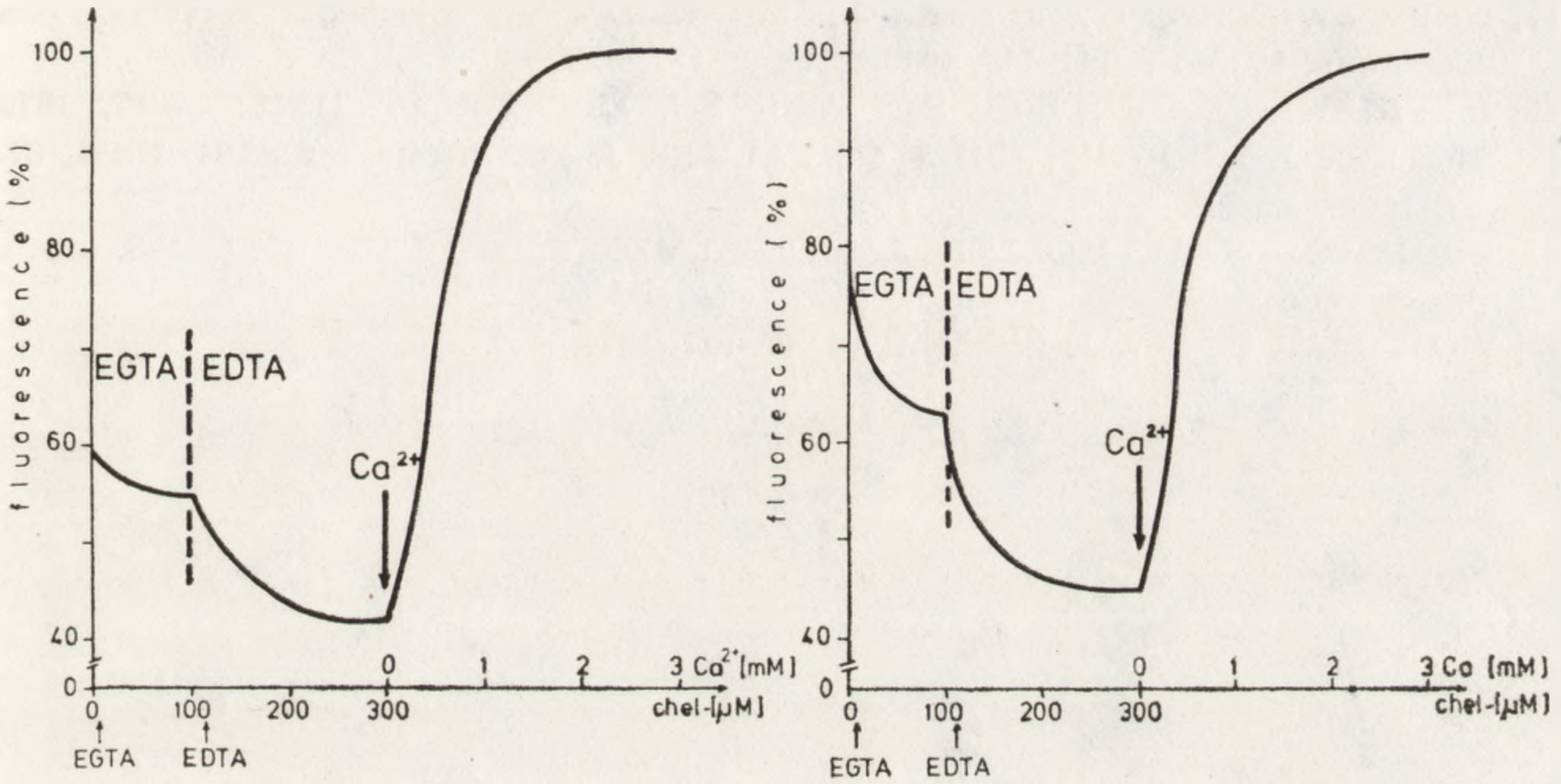

Fig. 3. Effect of EGTA, EDTA and Ca^{2+} on the relative intensity of 9-AA fluorescence

Mitochondria isolated: *A* — in standard conditions, *B* — without chelators. Incubation medium (1.5 ml, 25°C) contained; 250 mM sucrose, 10 mM Tris/HCL pH 7.4, 0.5 μM FCCP, 1.8 mg mitochondrial protein. EGTA and, consecutively EDTA were added in four equal steps. Ca^{2+} was introduced in 0.33 mM steps. Maximal fluorescence intensity, obtained with saturating Ca^{2+} concentrations, reflecting full screening, was taken as 100%

or absence of CTAB. However, this amount of Ca^{2+} is high enough to support the full activity of NADH oxidation. On the other hand 9-AA fuorescence increases twofold following the addition of 3 mM Ca^{2+} which suggests that the screening of intact mitochondria is not saturated. However, 70 μM CTAB has any effect on neither the fluorescence in absence of chelators nor on the shape of the curve of chelators action (Fig. 3).

The results show that in amoeba mitochondria Ca^{2+} ions exert, in the presence of the chelators, a specific stimulatory effect on NADH

oxidation, regardless of the degree of screening, as they do in plant mitochondria [6]. However, no dependence of activation of NADH oxidation by Ca^{2+} on the functional state of the enzyme is observed, in contrast to plant mitochondria [6].

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (319—322)

ENERGY COUPLING, MEMBRANE POTENTIAL AND Ca^{2+} TRANSPORT IN MITOCHONDRIA OF SNAIL HEART

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Snail heart mitochondria (SHM) were reported to display oxidative phosphorylation [1], in contrast to snail hepatopancreas mitochondria [2]. The present studies aimed to examine more precisely the degree of coupling, the efficiency of Ca^{2+} uptake and its correlation with the membrane potential.

Hearts of 120 specimens of *Helix pomatia* were cellected, digested with nagarase and homogenized in mannitol yielding ~ 20 mg of mito-

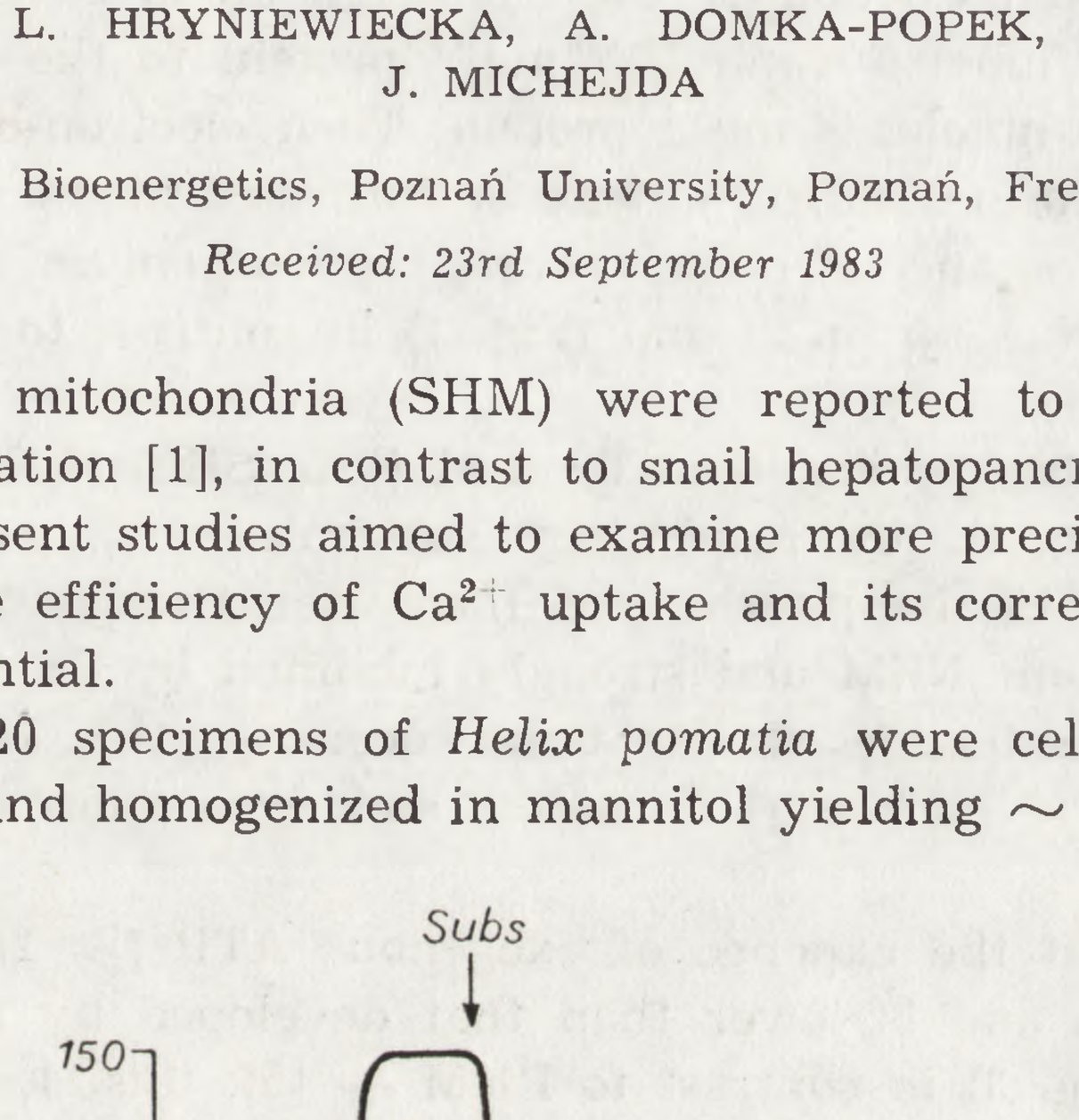

Fig. 1. Uptake of Ca^{2+} ions measured with Ca-electrode. Incubation medium (2 ml, 25°C): 115 mM sucrose, 20 mM P_i—buffer pH 7.4, 6 mM $MgCl_2$, 10 mM KCL, 0.2% BSA, 10 mM succinate; 2 µM ruthenium red (RR), 125 µM $CaCl_2$, 10–30 mM NaCl

chondrial protein. O_2 uptake was measured with the Clark oxygen electrode in 1.2 ml of the same medium as for Ca^{2+} uptake (Fig. 1).

In oxygraphic measurements SHM appear tightly coupled in respect to ADP and Ca^{2+}, 50 μM ADP and 300 μM Ca^{2+} saturating the respiratory control (3.6–5.8). About 2 Ca^{2+} is taken up for each phosphorylating site. Accumulation of $>$ 800 nmoles $Ca^{2+} \times mg^{-1}$ protein decreases the ADP:O ratio. Interestingly malate alone is oxidized linearly for a long time, though glutamate or pyruvate accelerate the respiration. Rotenone 10–40 μM decreases partially the oxygen uptake with substrates oxidized via NADH, while diS-C_3-(5) does not; both reagents, however, decrease ADP:O by about 30 percent. $^{45}Ca^{2+}$ is taken up in energized conditions at the rate 600 nmoles $\times$ $min^{-1} \times mg^{-1}$ protein to the level 150 ($-P_i$) and 1200 ($+P_i$) nmoles $\times$ mg^{-1} protein. Then electron-dense aggregates appear within mitochondria, absent in controles incubated with RR or FCCP. Slow spontaneous efflux caused by ruthenium red (RR) is not stimulated by Na^+ up to 30 mM (Fig. 1) in contrast to mammalian excitable tissues [4].

In spite of high endogenous level of P_i in SHM (100 nmoles $\times$ mg^{-1} prot.) $\Delta\psi$ supported by respiratory substrates requires exogenous P_i ($\geqslant$ 1 mM) and in the presence of the latter the generation of $\Delta\psi$ is deleyed by 20 μm NEM and strongly inhibited by $\geqslant$ 10 μm mersalyl.

Malate generates $\Delta\psi$ slower than succinate; this rate is increased by oligomycin (6 μg $\times$ mg^{-1} protein) and even more by RR (1 μM) (Fig. 2).

$\Delta\psi$ formed at the expence of exogenous ATP ($\geqslant$ 200 μM) requires Mg but not P_i, and is lower than that developed by respiratory substrates $+P_i$ (Fig. 3) in contrast to RHM — [5]; this $\Delta\psi$ is abolished by oligomycin or CAT (20 μM).

Malate generates $\Delta\psi$ regardless the presence of rotenone (15–40 μM) sugesting that rotenone and diS-C_3-(5) which uncouple the I site of phosphorylation do not block the transport of electrons, leaving site II and III operating. However, the low value of $\Delta\psi$ following RR addition in absence of exogenous substrates is abolished by 5 μM rotenone (Fig. 2).

Addition of Ca^{2+}, depending on its amount results in transient or permanent abolishment of $\Delta\psi$. Ommission of Mg^{2+} decreases the rate and the extent of $\Delta\psi$ (Fig. 3). In this case, however, following the accumulation of some amounts of Ca^{2+}, ATP is not able to generate $\Delta\psi$ in contrast to RHM [5]. Probably Ca^{2+} accumulation increases the efflux of Mg^{2+} from mitochondria [6].

In conclusion: SHM are tightly coupled, efficiently accumulate Ca^{2+} and generate $\Delta\psi$ by respiratory substrates (even in the presence of

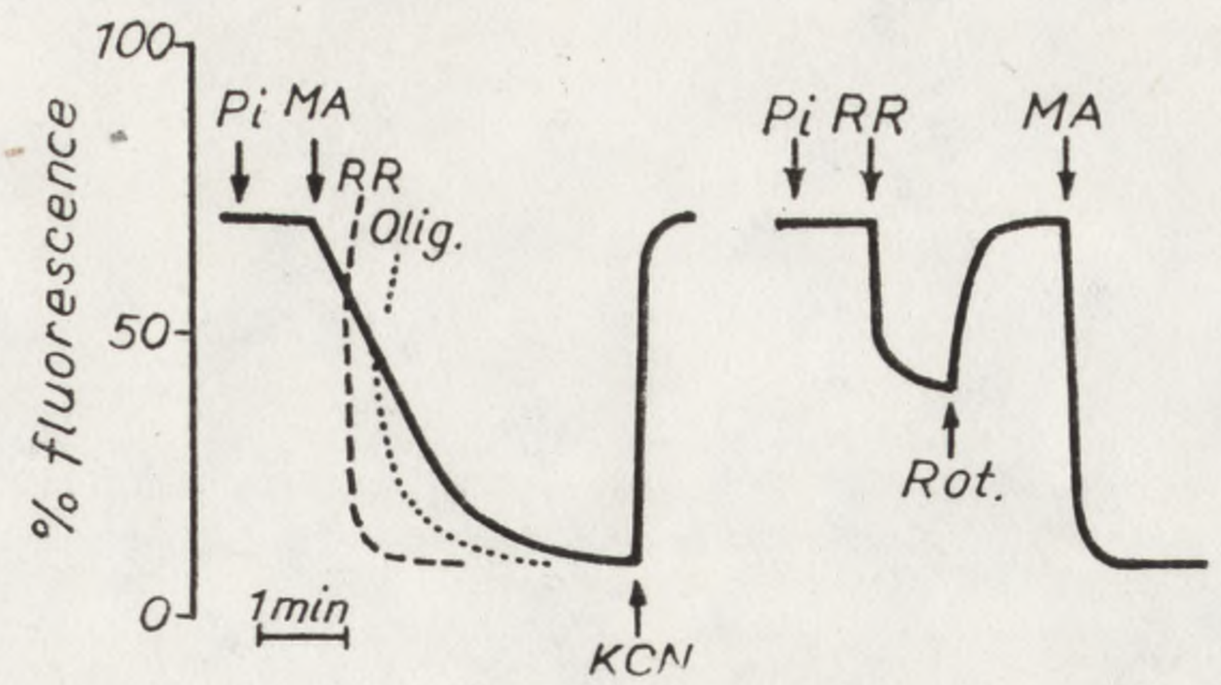

Fig. 2

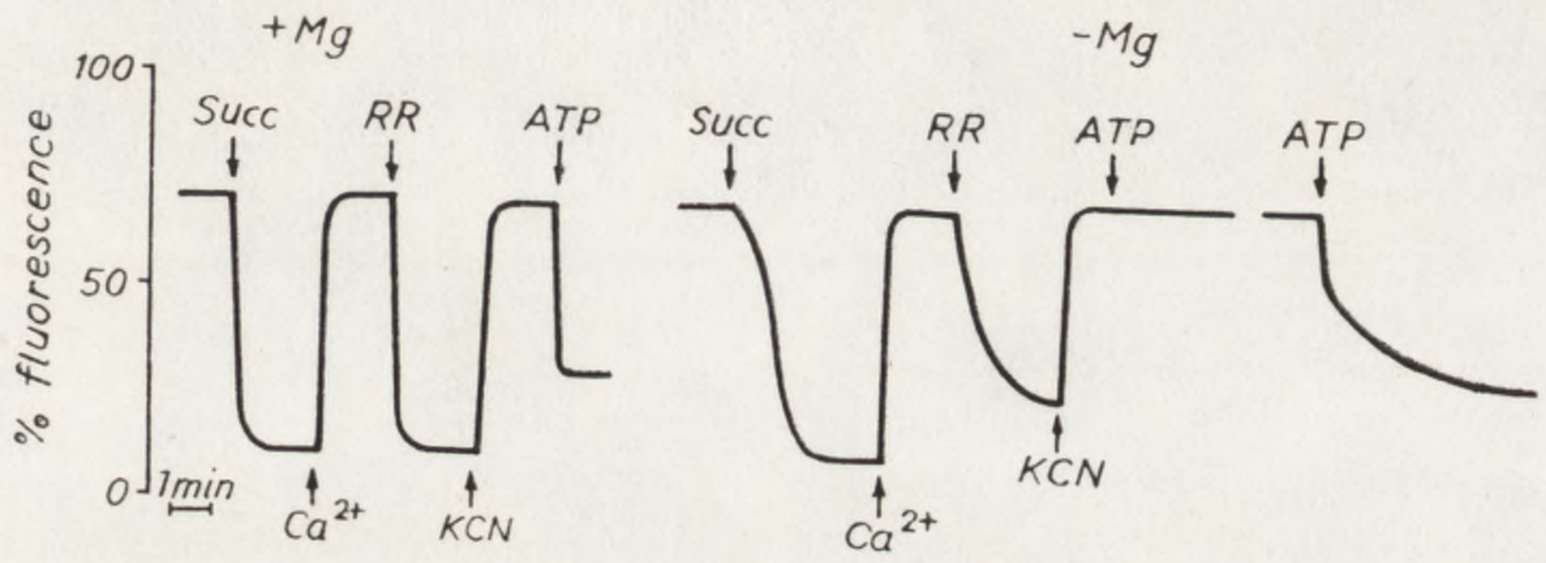

Fig. 3

Fig. 2 and 3. Changes in fluorescence intensity reflecting changes in $\Delta\psi$. Incubation medium (1.4 ml, 25°C): 225 mM sucrose, 4 mM TRA pH 7.4, 5 mM $MgCl_2$, 20 mM KCl, 0.4% BSA, 1.8 μM diS-C_3-(5), 0.3 mg mitochondrial protein. Additions: 2 mM P_i, 2 mM malate (MA), 1 μM ruthenium red (RR), oligomycin (6 μg/mg prot.), 1 mM KCN, 5–20 μM rotenone (rot), 2 mM succinate, 0.4 mM Ca^{2+}, 0.4 mM ATP

rotenone) and by ATP. The main leak in $\Delta\psi$ generation is the cycling of Ca^{2+}, although not reflected in oxygen uptake. No Na^+/Ca^{2+} antiport seems to exist in this excitable invertebrate tissue.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (323—326)

ACTIVATION OF ATPase DUE TO PREENERGIZATION IN AMOEBA MITOCHONDRIA

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It is generally accepted that mitochondrial ATPase is physiologically regulated by native peptidic inhibitor integral in the ATPase [1, 2]. In mammalian mitochondria the inhibitor dissociates from the ATPase as the effect of energizing preincubation, unmasking full activity of

TABLE 1

Effect of preincubation of mitochondria in energizing conditions on ATPase activity Incubation medium (0.4 ml, 28°C) contained: 20 mM Tris-HCl buffer pH 7.4, 140 mM sucrose, 5 mM ATP, 2.5 mM $MgSO_4$, 5 mM PEP, 11 u × mg^{-1} mito protein, 400 μg mito protein. Additions: 1.4 mM NADH or 10 mM succinate. Activity was measured for 5 minutes, and is given in nmoles P_i × mg^{-1} protein × min^{-1}. Preincubation (5 minutes) prior to addition of ATP. Percent of stimulation in presence of CN+SHAM was calculated assuming activity 28 as 100%.

Specification	Inhibitor added		Specific activity	Stimulation in %
	before substrate	together with ATP		
None	—	—	42	—
NADH	FCCP	—	43	
	antimycin or CN	—	40	
	CN+SHAM	—	28	
NADH	—	—	85	107
	—	FCCP	56	36
	—	antimycin or CN	80	100
	—	CN+SHAM	49	75
Succinate	—	—	71	73
	—	FCCP	55	34
	—	CN	73	78

ATPase in a hydrolitic, and probably synthetic direction [3, 4]. Activation of ATPase due to energizing conditions was also visualized as the increase in the rate of Ca^{2+} ions uptake supported solely by ATP [5]. So far no information is available on the native ATPase inhibitor in Protozoan mitochondria or on the effect of preenergization on their ATPase activity. However, such activation was reported in plant mitochondria which share many features in common with amoeba mitochondria. Mitochondria of *Acanthamoeba castellanii* reveal a considerable ATPase activity [6] and are able to generate $\Delta\psi$ [7] and to support Ca^{2+} uptake [8].

Activity of ATPase was measured as the liberation of inorganic phosphate in a system regenerating ATP (phosphoenolopyruvate and pyruvate kinase) [6]. Mitochondria were prepared from trophozoites from an exponential and stationary phase of growth [6]. Energization was performed by 5-minute incubation with NADH or succinate, the effective respiratory substrates [9]. In tritonized or sonicated mitochondria the controlled digestion by tripsin yields twofold activation of ATPase activity, regardless of the phase of cultures from which mitochondria were isolated. Preincubation for 5 minutes in the energizing conditions increases the ATPase activity of intact mitochondria almost twofold, in the presence of 2.5 mM Mg^{2+} (Table 1). Without the respiratory substrates the ATPase activity does not depend on the presence

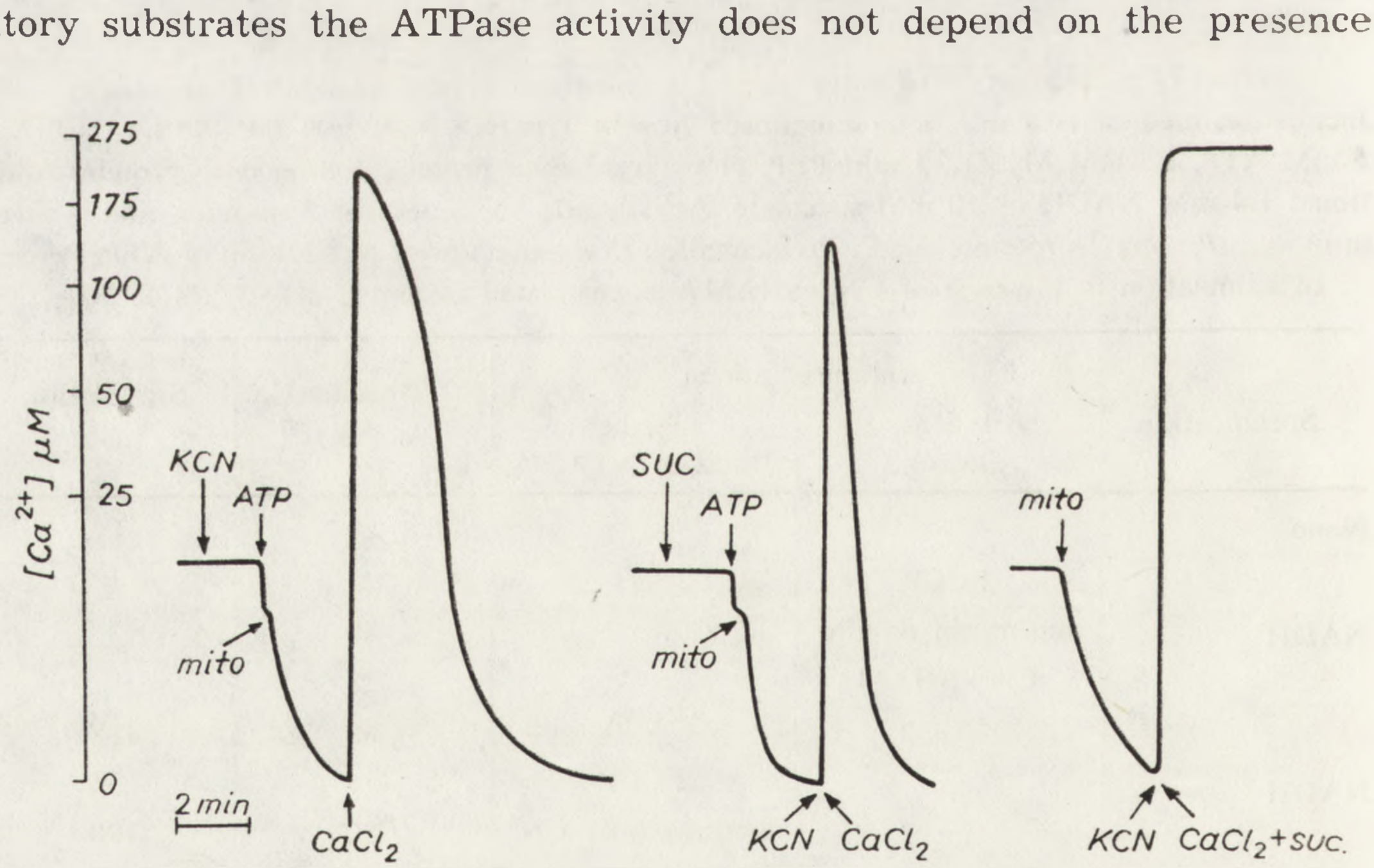

Fig. 1. Effect of the preincubation with succinate on ATP-supported Ca^{2+} uptake by mitochondria. 2 ml medium contained: 120 mM KCl, 20 mM Tris-HCl pH 7.4, 3 mM KH_2PO_4, 8 mM $MgCl_2$, 0.2% BSA, 1.7 mg mitochondrial protein; 25°C. Additions: 250 µM $CaCl_2$, 5 mM succinate, 1 mM KCN, 5 mM ATP

of respiratory inhibitors or FCCP applied either together with ATP or 5 minutes earlier. The activity is not increased when these inhibitors are added neither previously to the substrates, nor instead of substrates. However, the addition of inhibitors (antimycin or CN) together with ATP, following a 5-minute preincubation with the respiratory substrate, results in a twofold increase in the ATPase activity. On the other hand, the abolishing of energization by FCCP yields much lower stimulation. When energization is stopped 1 minute prior to ATP addition, the stimulation of ATPase activity is decreased by 70%, while in the analogous conditions $\Delta\psi$ was abolished in less than 30 seconds [7]. In the presence of Mg^{2+}, FCCP does not stimulate amoeba ATPase but it does it in the absence of Mg^{2+}. When preincubation in energizing conditions is carried out in the absence of Mg^{2+}, the activity of ATPase is several times higher in the presence of FCCP, than in the presence of antimycin. 0.2% BSA potentiates the stimulatory effect of FCCP on the ATPase activity.

The rate of Ca^{2+} uptake measured with Ca^{2+}-selective electrode [10] supported solely by ATP is also significantly increased by a 2-minute preincubation in energizing conditions (Fig. 1). This Ca^{2+} uptake is totally blocked by oligomycin. Energizing preincubation had to be carried out, however, in the presence of both, succinate and ATP, because if ATPase is not operating from the beginning of the preincubation, the later addition of ATP does not promote any Ca^{2+} uptake. Similar activation is obtained when ATP is the only preenergizing agent for at least 1 minute. The results suggest the regulatory role of the native ATPase inhibitor in amoeba mitochondria.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (327—330)

Ca^{2+} UPTAKE IN AMOEBA MITOCHONDRIA SUPPORTED BY CYANIDE-INSENSITIVE RESPIRATION AND BY ATP

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Mitochondria of the soil amoeba, *Acanthamoeba castellanii* Neff are tightly coupled when respiring via the cytochrome pathway [1, 2]. Under these conditions the mitochondria reveal a high capacity for $^{45}Ca^{2+}$ uptake [3] and their respiration is stimulated by Ca^{2+} ions, [2]. Moreover, mitochondria of *A. castellanii*, especially those from the exponential phase of culture growing in the presence of Fe, show a high activity of the alternative respiratory pathway [4, 5].

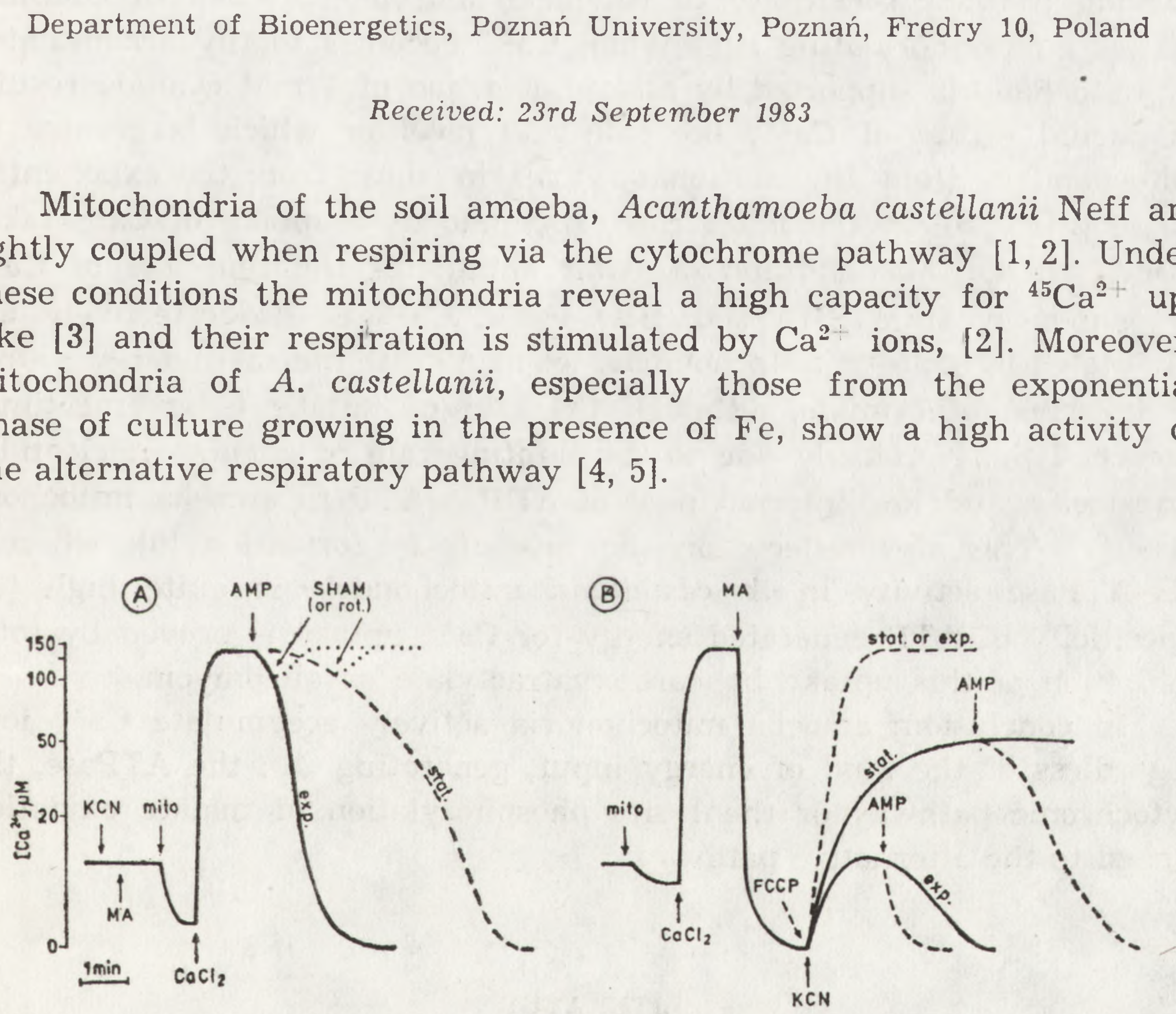

Fig. 1. Uptake (*A*) and efflux (*B*) of Ca^{2+} ions monitored ba Ca-selective electrode in mitochondria of *A. castellanii* from the stationary or exponential phase of culture. 2 ml medium contained: 120 mM KCl, 20 mM Tris HCl pH 7.4, 3 mM KH_2PO_4, 8 mM $MgCl_2$, 0.2% BSA, 1.3 mg mitochondrial protein; 25°. Additions: 125 μM $CaCl_2$, 5 mM malate, 1 mM KCN, 2 mM AMP, 1 mM SHAM, 10 μM rotenone, 10 μm FCCP

The rate of Ca^{2+} uptake by amoeba mitochondria supported by malate oxidized in the absence of pyruvate and cyanide is similar to that supported by succinate. However, in the presence of cyanide no uptake of Ca^{2+} is observed until AMP (1 mM) is added (Fig. 1*A*). The response is faster in mitochondria isolated from the exponential phase of growth, than in those from the stationary one. 1 mM salicylhydroxamic acid (SHAM) or 10 μM rotenone abolish Ca^{2+} uptake under these conditions. This phenomenon corresponds to the changes in $\Delta\psi$ generated by malate (+AMP) in presence of cyanide, and abolished by the addition of Ca^{2+} ions [6]. In the absence of cyanide, rotenone reduces only the rate of but not the extent of Ca^{2+} uptake, supported by malate. The results suggest the presence of two kinds of NADH dehydrogenases varying in their sensitivity to rotenone and in efficiency of coupling at the I phosphorylating site. When Ca^{2+} becomes totally accumulated by mitochondria supported by malate, addition of 1 mM cyanide results in partial efflux of Ca^{2+}, the rate and level of which is greater in mitochondria from the stationary than in those from the exponential phase (Fig. 1*B*). In the latter case, spontaneous reuptake of Ca^{2+} takes place, whereas the addition of AMP stimulates the reuptake of Ca^{2+} prevented by 1mM SHAM in both cases. Ca^{2+} is also effectively accumulated by amoeba mitochondria, when ATP is the sole energy source in presence of cyanide, although the rate of uptake is several times slower. This is probably due to the limiting rate of adenine nucleotides translocase and low internal pool of ADP + ATP in amoeba mitochondria [7]. This also reflects on the rate of $\Delta\psi$ formation [6], whereas Mg-ATPase activity in *A. castellanii* mitochondria is quite high [8]. Specificity of ATP generated energy for Ca^{2+} uptake is proved by total inhibition of this uptake by carboxyatractylate or oligomycin.

In conclusion: amoeba mitochondria actively accumulate Ca^{2+} ions regardless of the kind of energy input, generating $\Delta\psi$: the ATPase, the cytochrome pathway or the I site phosphorylation of malate oxidation linked to the alternative pathway.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (331—334)

PATHWAYS OF MALATE OXIDATION IN MITOCHONDRIA OF *ACANTHAMOEBA CASTELLANII* DURING STATE 4 RESPIRATION

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Mitochondria of the amoeba *Acanthamoeba castellanii* Neff are tightly coupled [1] and reveal the presence of both the cytochrome and the alternative respiratory pathway [2, 3]. Oxygen uptake supported by malate is only partially sensitive to rotenone [1] and is capable of generating $\Delta\psi$ even in the presence of cyanide, when supported by AMP [4]. Malate is an efficient substrate in amoeba mitochondria and, like in plant mitochondria [5], does not require exogenous pyruvate (Fig. 1*a*). State 4 respiration in mitochondria from the exponential phase of culture is inhibited by cyanide only to 60–40 percent, while regard-

Fig. 1. Oxygraphic traces of oxygen uptake by amoeba mitochondria. Incubation medium (1.5 ml) contained: 120 mM KCl, 20 mM Tris-HCl pH 7.4, 6 mM $MgCl_2$, 8 mM KH_2PO_4, 0.2% BSA, 1.5 mg mito. protein; 25°. Additions: 2 mM malate (MA), 1 mM KCN, 1 mM NAD^+, 1 mM NADH, 3 mM AMP, 1 mM SHAM, 60 mM butylmalonate (BM), 10 mM oxaloacetate (OAA), 160 μM ADP. Values represent $nAtO_2/min \cdot mg$ protein

TABLE 1

Products of malate oxidation in amoeba mitochondria. Conditions of 4 min. incubation as in Fig. 1, except for 3 mM arsenite and 5 mg mito. protein. Oxaloacetate (OAA) and pyruvate (PA) were assayed enzymatically in PCA extracts of the supernatant (s) and mitochondrial pellet (p). Amounts of malate (MA) oxidized calculated in nmoles/min·mg protein from O_2 uptake. Other values given in percent of MA oxidized

Additions	MA	PA		OAA		OAA/PA
		s	p	s	p	
nil.—ars.	43	7	1	1	0	0.1
nil.	32	34	19	1	1	<0.1
CN	7	34	61	0	2	<0.1
CN+AMP	13	8	17	10	5	0.6
CN+NAD^+	16	16	50	20	3	0.3
CN+NAD^+, —Mg	15	14	24	30	7	1.0

less of the presence or absence of cyanide it is significantly stimulated by NAD^+ (or by AMP) to 120–150 percent of the original rate (Fig. 1*b*). In the presence of KCN the respiration is completely abolished by salicylhydroxamate (SHAM) and strongly inhibited by butylmalonate, an inhibitor of dicarboxylate transport. Butylmalonate does not inhibit the oxygen uptake supported by exogenous NADH (Fig. 1*c*). The stimulation of this respiration in the presence of KCN by oxaloacetate being only twofold in contrast to plant mitochondria [5] is abolished by butylmalonate. Arsenite decreases the rate of respiration, increases its inhibition by cyanide and decreases its stimulation due to NAD^+ or AMP.

Enzymatic assay of malate oxidation products in state 4 in the presence of arsenite (Table 1) revealed that regardless the native cooperation of both pathways (—KCN) or the exclusion of the cytochrome pathway by KCN, the main product is pyruvate. However, when the alternative pathway (+KCN) is supported by NAD^+ or AMP a considerable amount of oxaloacetate appears and hence the OAA : PA ratio increases. Omission of Mg results in a further shift in favour of OAA produced. Significantly more pyruvate is formed in the presence of NAD^+ than in the presence of AMP. When only the alternative pathway operates more of the pyruvate formed is retained within mitochondria, suggesting that lower $\Delta\psi$ [4] hinders the efflux of pyruvate. In mitochondria a moderately active malate dehydrogenase was found together with a more active NAD^+-dependent malic enzyme, stimulated by Mg and Mn ions.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (335—338)

PRELIMINARY CHARACTERIZATION OF SARCOPLASMIC RETICULUM SR AND SARCOLEMMA SL FROM RABBIT STOMACH SMOOTH MUSCLES

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Contrary to skeletal muscles, smooth muscle cell does not contain typical intracellular membranous structure specialized in the transport and accumulation of Ca ions. The ratio of plasma membrane to SR in smooth muscle is much higher than that of skeletal muscle, i.e. a large part of the microsomal vesicles of smooth muscle may originate from the plasma membrane [1]. It is not clear which membranous systems are responsible for regulation of calcium concentration in contraction--relaxation cycle of smooth muscle. The aim of the present study was to purify and to characterize the sarcoplasmic reticulum (SR) and the sarcolemma (SL) vesicles from stomach smooth muscle and to provide positive identification of the constituents of these membranes as well as of contamination by membrane of other organelles.

The stomach from albino rabbits (2.5–3.0 kg) was excised and fat was removed. The smooth muscle layers from the stomach antrum were separated from the mucous layer. Preparation of SR fraction was performed as described previously [1], except that loading with calcium phosphate was omitted. Preparation of SL fraction was carried out as described for rat myometrium [2], except that muscle homogenization was done in 0.25 M sucrose, 20 mM TRIS-HCl, pH 7.2, 0.1 M KCL, and 2 mM DTT, using Warying-blendor. Activities of marker enzymes (5′-nucleotidase, NADH- and NADPH-cytochrome c reductase, Na^+, K^+-ATPase, and Ca^{2+}, Mg^{2+}-ATPase) were assayed as described previously [3]. Lysolecithin acyltransferase and cholinephosphotransferase activities were measured according to [4, 5], respectively. Cholesterol was extracted according to [3], than sililated using N,O-Bis (Trimethylsilyl)-trifluoro-

TABLE 1

Distribution of marker enzymes activities in SR and SL fractions from stomach smooth muscle

Fraction	Mg^{2+}-dependent ATPase	Ca^{2+}-stimulated ATPase	Na^{+}, K^{+}-ATPase	5′-nucleotidase	NADPH-cytochrome c reductase*	NADH-cytochrome c reductase
	μmoles /mg of protein/ minute					
SR	0.81	0.36	0.02	0.01	11.6	0.12
SL	1.69	0.14	0.27	0.12	8.0	0.04

* nmoles/mg of protein/minute

TABLE 2

Lipid composition and activities of phospholipid synthetizing enzymes in SR and SL fractions isolated from stomach smooth muscle

Fraction	total lipid / protein	phospholipid / protein	PE and PC content % of total PL		cholesterol / protein	lysolecithin acyltransferase		cholinephospho-transferase	
	μmoles/mg		PE	PC	μg/mg	nmoles/mg/15 min			
SR	0.98	0.59	15	49	100	8.2^{+}	4.1^{++}	20.4$^{=}$	3.0$^{==}$
SL	1.15	0.61	18	48	170	32.8^{+}	6.2^{++}	5.1$^{=}$	0.8$^{==}$

Abbreviations used: PL — phospholipids; PC — lecithin phosphatidylcholine; PE — phosphatidylethandamine
= + egzogenous 1,2-diacylglycerol; = = — egzogenus 1,2-diacylglycerol;
+ + egzogenous lysolecithin; + + — egzogenous lysolecithin

acetamide (BSTFA), and analysed using gas liquid chromatography (G.L.C.) with cholestan as an internal standard. Lipids were extracted by the Bligh and Dyer's method, and separated using thin-layer chromatography as described previously [3]. Fatty acids from total lipids or phospholipids were methylated using boron fluoride in 14% methanol, and analysed by G.L.C. technique with pentadecanoic acid as an internal standard.

After homogenization of stomach smooth muscle, the microsomal fractions were sedimented between 10 000 × g and 100 000 × g. The major constituent of crude microsomal fraction (100 000 × g sedimenting pellet) are sarcolemma vesicles, and only few percent of this fraction accounts for SR, as examined by electron microscopy technique. The microsomal fraction consisting of vesicles of various size and density was separated on a step sucrose density gradient into four subfractions containing SL, heterogenous vesicles, SR and probably mitochondrial membrane fragments, respectively. Purity of these subfractions was checked by measuring 5′-nucleotidase and Na^+, K^+-ATPase, NADPH--cytochrome c reductase, Ca^{2+}, Mg^{2+}-ATPase, and NADH-cytochrome c reductase activities as marker enzymes for SL, SR and outer mitochondrial membrane, respectively.

5′-nucleotidase and Na^+, K^+-ATPase appear to be a predominantly plasma membrane-bound enzymes [6]. As shown in Table 1, their activities are about 12 and 14 times higher in SL than in SR. The activities of NADPH-cytochrome c reductase and Mg^{2+} -dependent ATPase do not differ significantly in both fractions. Biochemical analysis of marker enzymes excluded minor contamination of SR and SL preparations by outer mitochondrial membrane, because 5 mM NaN_3 had no inhibitory effect on Mg^{2+}-dependent ATPase activity, and we observed relatively low activity of rotenone insensitive NADH-cytochrome c reductase in these fractions.

Both fractions, SR and SL, reveal the activity of Ca^{2+}-stimulated ATPase — the enzyme responsible for the regulation of Ca ions concentration in cytoplasm. However, because in smooth muscle cell SL is the predominant membranous system, thus probably it plays the essential role in the regulation of Ca ions concentration.

The important role of lipids in membrane function is well documented in skeletal muscles, but there is no data available on lipid content, composition and metabolism in microsomes isolated from smooth muscle. Differences in total lipids and phospholipids content between SL and SR in smooth muscle are not significant, only cholesterol to protein ratio is higher in SL than in SR. Biosynthesis of phospholipids occurs in microsomal fractions isolated from smooth muscle as well as in ske-

letal muscle. Like in skeletal muscle, where cholinephosphotransferase activity is predominantly bound with SR membrane, and lysolecithin acyltransferase with SL membrane [4, 5], we have shown that the same situation exists in smooth muscle (see Table 2), however the activities of both enzymes were lower in smooth muscle than in skeletal muscle.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (339—340)

EFFECT OF QUENCHING OF CDC-DERIVED MEMBRANE FLUORESCENCE

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Unicellular protozoan cell *Paramecium aurelia* (*Ciliata*) can be supravitally labelled with cycloheptaamylose-dansyl chloride complex (CDC) as a fluorescent marker of the surface membrane [1, 2].

The long-term starved cells were labelled with fluorochrome to avoid the influence of autofluorescence. When dried samples of the CDC-labelled cells have been irradiated with UV the green fluorescence of the membrane (with maximum at 460 nm) gradually photofaded and the small orange fluorescent structures appeared intracellularly (with broad emission peak at about 570 nm).

Microspectrofluorimetric analysis indicated that the emission spectra of these unknown structures are different from those observed in nonstained autofluorescent cells. The latter ones show a faint yellowish-green fluorescence. Observations carried out on the ciliates which had uptaken polystyrene beads prior to CDC labelling demonstrated that orange "bodies" do not appear in these cells after UV irradiation.

Experiments on *Paramecium cells* exhibiting altered phagocytic activity indicate that the appearance of intracellular orange fluorescence is due to uptake of minimal quantities of CDC occurring during labelling of the living and long-term starved cells. However, the chemical basis of the reaction leading to the emission in this spectral region is not yet clear. Stimulation of phagocytosis resulted in a greater amount of orange structures whereas its inhibition — in a complete disappearance. The effectiveness of stimulation and inhibition of phagocytosis

was examined in an independent experiment using fluorescent polystyrene beads and their uptake was observed. Thus it can be concluded that appearance of intracellular orange fluorescence is directly dependent on the capacity of the cells to endocytosis. The obtained results indicate that non-penetrating surface markers may be — under some experimental conditions — uptaken to the cell via endocytosis.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (341—342)

CALCIUM-PHOSPHORUS-CONTAINING STRUCTURES AND Ca-ATPase AT THE PLASMA MEMBRANE OF *ACANTHAMOEBA CASTELLANII* AND THEIR RELATION TO PHAGOCYTOSIS AND PSEUDOPODIAL REACTION

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Acanthamoeba castellanii cells fixed with either glutaraldehyde or formaldehyde in the presence of 1–15 mM $CaCl_2$ displayed numerous electron-dense structures which were localized only in local areas at the cytoplasmic side of plasma membrane. The similar structures appeared when the cells were fixed by quick-freezing and subsequently freeze-substituted. The appearance of the electron-dense structures was not enhanced by replacement of Ca by either Na, K, Mg or La. X-ray microprobe analysis revealed calcium and phosphorus as major elements of the structures [1, 2]. Calcium-phosphorus-containing electron-dense structures (CPS) had ellipsoidal form and their longitudinal diameter was ca. 90 mm when the cells were fixed with glutaraldehyde, and ca. 40 nm after fixation with formaldehyde. Measurements of the CPS frequency showed that in the glutaraldehyde fixed cells 3.7 CPS per 1 μm of membrane profile could be seen, whereas after formaldehyde fixation the frequency of CPS was twice increased (Table 1). The presence of Ca-ATPase activity at the same places of membrane and with

TABLE 1

Frequency and diameter of CPS and Ca-ATPase products

Fixative	Ca-phosphorus structures		Ca-ATPase	
	Frequency (CPS/μm)	Diameter (nm)	Frequency (product/μm)	Diameter (nm)
Glutaraldehyde	3.7	92.6	3.4	86.6
Formaldehyde	7.8	42.1	6.8	47.4

the same frequency as for CPS was detected by cytochemical method (Table 1). The enzyme showed high activity in the presence of calcium ions and ATP as substrate.

The granular products of Ca-ATPase activity and CPS were usually abundant at the plasma membrane of pseudopodia and those taking part in phagocytic cups formation. Ca-ATPase activity was significantly inhibited by calmodulin-inhibitors such as trifluoperazine and chlorpromazine. The drugs abolished also the formation of CPS (about 40%) and inhibited also the process of phagocytosis. Treatment of CPS with exogenous alkaline phosphatase induced partial clearing of the electron-dense structures resulting in appearance of delicate, well organized fibrinous material inside CPS. These results indicate that CPS represent the phosphorylated compounds with high affinity to calcium ions forming electron-dense structures which could be partialy decomposed by phosphatase.

The present study suggests that at the plasma membrane of *Acanthamoeba castellanii* the calcium-phosphorus-containing structures and Ca-ATPase form a functional complex which may be under the calmodulin control, and indicates that these structures play a role in phagocytosis and pseudopodial activity.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (343—344)

INFLUENCE OF CALMODULIN-INHIBITORS ON PHAGOCYTOSIS IN AMOEBA

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Acanthamoeba castellanii is a convenient model in experiments on phagocytosis. This process is known to be under calcium control and probably depends on calmodulin activity. There are some drugs known as the calmodulin inhibitors, e.g. trifluoperazine (TFP), chlorpromazine, reserpine [3].

The study was carried out on *Acanthamoeba castellanii* and on *Amoeba proteus*. The phagocytosis was tested by using either fresh human erythrocytes or *Saccharomycetes cerevisiae*. For examination of calmodulin-inhibitors action on phagocytosis *Acanthamoeba* cells were preincubated with TFP or chlorpromazine or reserpine. The uptake of erythrocytes by the drug-treated cells was significantly inhibited (Table 1), whereas the phagocytosis in untreated amoebae was linear for about 20 min.

TABLE 1

Name of applied drugs	% of phagocytosis inhibition at different time	
	10 min	20 min
TFP (10 μM)	32	42
Chlorpromazine (100 μM)	82	87
Reserpine (200 μM)	56	68

The inhibition of phagocytosis by the drugs is probably provoked by calcium replacement from its membrane binding sites. The membrane-associated calcium may be visualized by chlortetracycyline (CTC), which is known as a good fluorescent calcium-probe [1]. The brightness of CTC-fluorescence depends on calcium content in membranes [2].

When *Amoeba proteus* was stained with 350 μM CTC, pH 7.2, in a growth medium, bright fluorescence of plasma membrane region was visible. Addition of reserpine in low concentration (1 μM) or chlorpromazine (10 M) to the fluorescent samples caused the fading of fluorescence, what suggests that calmodulin-inhibitors displace calcium from its binding sites. In the plasma membranes nucleoside phosphatase is also localized — the enzyme which may be involved in process of phagocytosis [4]. *Acanthamoeba* cells were pretreated with 20 μM TFP or 100 μM chlorpromazine before fixation with glutaraldehyde, and these drugs were present in incubation media during all Ca-ATPase detection procedure. The Ca-ATPase activity was completely inhibited by the used drugs. These results indicate that the process of phagocytosis is related to the presence of an adequate level of calcium in the plasma membrane and to the activity of the membranous Ca-ATPase, which seems to be under calmodulin control.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (345—348)

HUMAN PLACENTAL BARRIER STUDY USING FREEZE-FRACTURING TECHNIQUE

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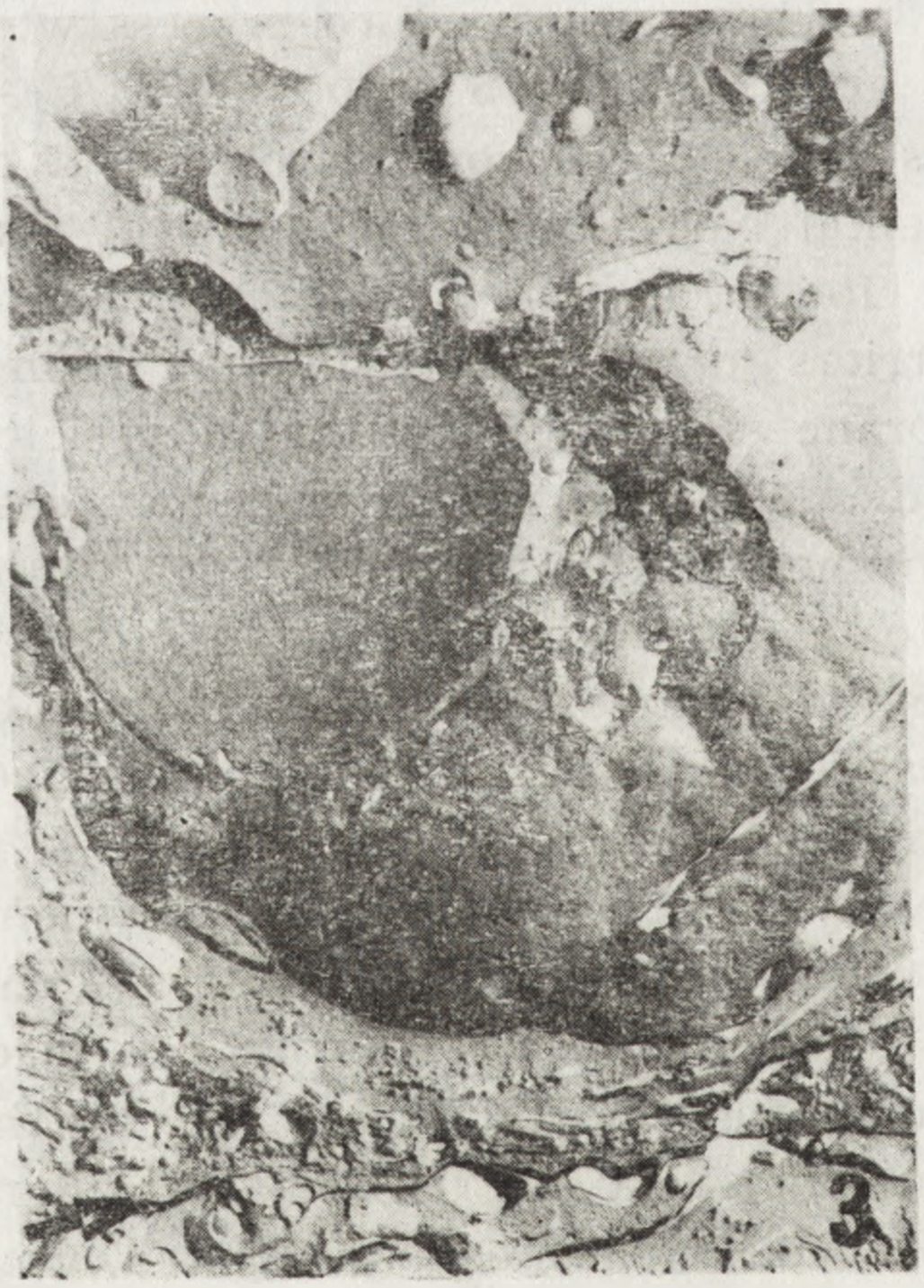
The aim of this study was the presentation of the structure and function relation of the placental barrier membranes in human.

Specimens (± 1mm^3) were dissected out from full-term placentas and prepared in a routine way [2, 3]. The freeze-fracture process were performed in an Edwards 306 Unit at −100°C at 5×10^{-7} T. Pt/C — replicas were examined with Philips EM 300 electron microscope at an accelerating voltage of 80 kV. Placental barrier which consists of syncytiotrophoblast, basal lamina and foetal capillary endothelium seems to be an example of other blood-barriers in man. The syncytial trophoblast: Cytoplasm was rich in membrane structures; system of vesicles, cysterns and channels of different diameter and length is clearly seen (Figs. 1, 2). Between parallel membranes in the interior of syncytium and invaginations of maternal and foetal surface different types of cell functions were observed. Transsyncytial channels and cisterns can be interpreted as a second way of exchange, particularly soluble substances of low molecular weight, between maternal and foetal blood [1, 2, 4, 6, 7]. Basal lamina which consists of syncytial and endothelial basal membranes is, in contrast to observation using conventional methods of TEM, amorphous with fractured colagen fibers (Figs. 1–3). This part of placental barrier similarly as other human functional barrier may be the most important element of this functional unit [1, 2]. Foetal capillary endothelium is usually built up few endothelial cells tightened with tortuous intercellular junctions. Distinct parts have been recognized: (a) the parajunctional zone with a low frequency or absence of plasmalemmal vesicles and other subcellular components; (b) the peripheral zone with large vesicle population which appears to be functionally the



Figs. 1-4

most important part of the endothelial cell; (c) the organelle region — generally adjacent to the nucleus — with a high concentration of cytoplasmic organelles; and (d) the nuclear region-nucleus and a thin shell of cytoplasm around it — usually devoid of cellular organelles. In the organelle and parajunctional regions microtubules and microfilaments are usually seen. Between adjoining endothelial cells extracellular channels are visible. It is also interesting that neighbouring cells differ in the composition of peripheral zone, particularly in the number and diameter of vesicles. We suggested that endothelial cells of foetal blood vessels works alternately (Fig. 3). Sometimes a single cytotrophoblastic cell can be recognized (Fig. 4) in the analysed region of placenta. This cell has not abundant organella and cellular membrane with characteristic digitate impressions.

This report has revealed that freeze-fracturing may be very useful in the study of human placental barrier layers. In reference to earlier data concerning human placental ultrastructure [2, 4, 6, 7] and to our previous studies [1] we are preparing an experiment to solve functional and structural relation problems in foeto-maternal exchange.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (349—352)

ELECTRON MICROSCOPIC STUDIES ON ULTRASTRUCTURAL MARKERS OF IMPULSE CONDUCTION IN THE SYNAPSES OF THE ACOUSTIC TRACT

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In order to determine impulse conduction in the synapses, it was decided to characterize energetical state of synaptic mitochondria by means of stereologic parameters. Electron microscopic studies were performed on the first synapse between the hair cells and the dendritic processes of the ganglion cells. The studies were carried out on 5 guinea pigs of both sexes, weighing 200–260 g. The animals were anaesthetized with Relanium• (5–10 mg) or Nembutal (30 mg/kg) given intraperitoneally. Then, the temporal bone was isolated and the pyramid of the temporal bone with the cochlea was exposed [1, 3]. After the cochlea exposure, its apical part and the secondary tympanic membrane in the fenestra rotunda were removed. The cochlea was perfused through its apical part with 5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4 by means of a thin polyurethane tube at a rate of approximately 0.1 ml/min. Then, the cochlea was broken off the temporal bone and fixed by immersion in the same fixative for 1 hour, washed in phosphate buffer and postfixed in 1% osmium tetroxide for 1 hour. After three washings in phosphate buffer for 15 min. it was dehydrated in ethanol and propylene oxide and embedded in Araldite [4–6]. The bone excess was removed with a razor blade under a binocular magnifying glass in order to obtain the cochlea alone, which subsequently was embedded again in fresh Araldite, transversely along the axis of the block. The obtained blocks with the embedded cochlea were placed in a LKB III holder and the osseous part of the cochlea was removed. Ultrathin sections were examined by means of a Philips EM 300 electron micro-

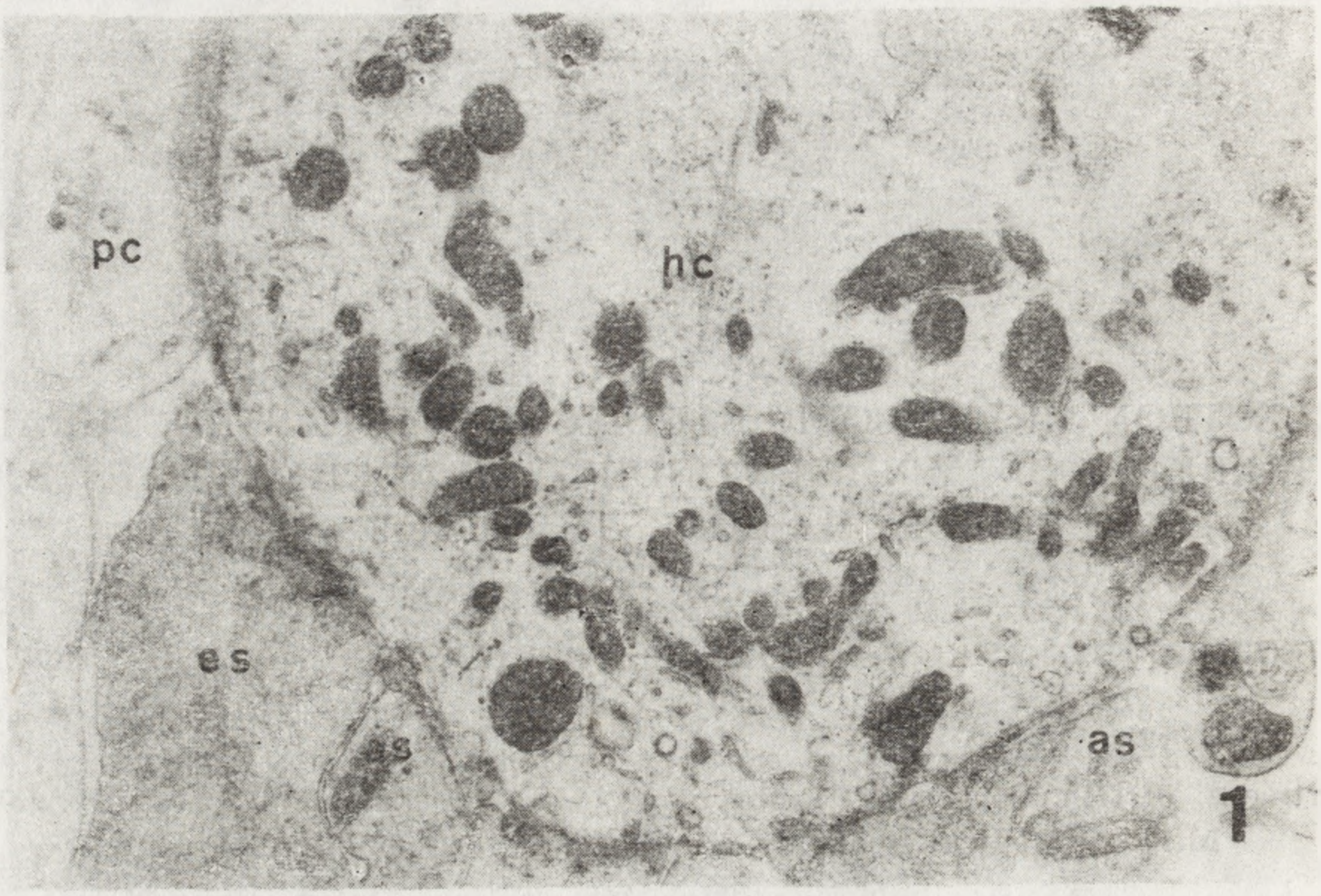

Fig. 1

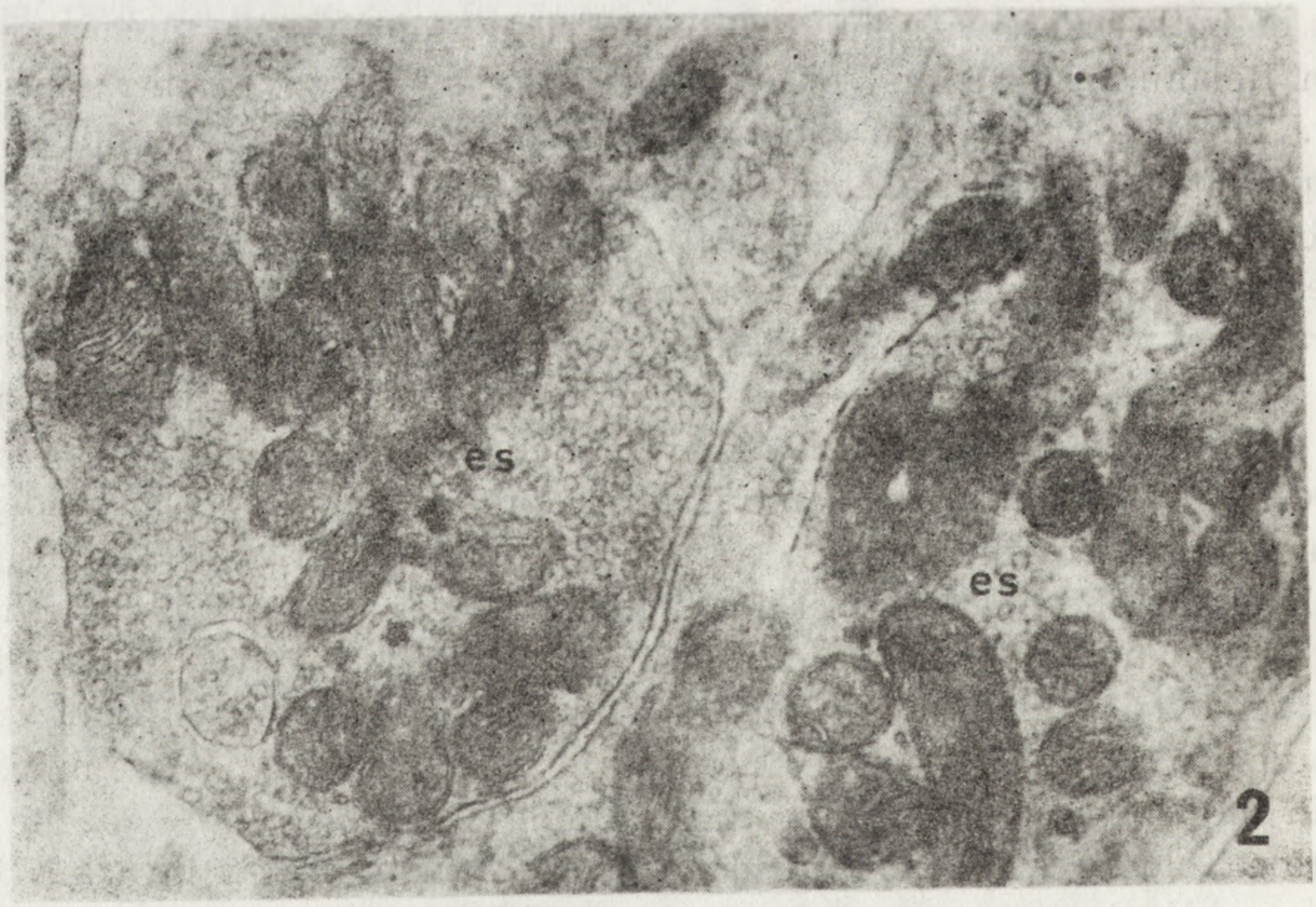

Fig. 2

scope. On the borderline between the hair cell and the dendritic processes of the ganglion cells a few separate synapses were observed. The afferent and efferent synapses could be distinguished (Figs. 1, 2: as — afferent synapse; es — efferent synapse; hc — the hair cell; pc — the phalangeal cell). The efferent synapse was characterized by large amounts of mitochondria and synaptic vesicles as opposed to the afferent ones, which contained significantly fewer amounts of these organellae. The cytoplasm of the afferent synapse was brighter and its synaptic vesicles were very diversified in respect to their shape and size. The pre- and postsynaptic membranes were darkened in both types of the synapses.

The hair cell pole adjacent to the dendritic synapses contains numerous mitochondria with microtubules between them. The visual evaluation of the hair cell mitochondria and synapses revealed that the ciliated cell mitochondria were of smaller size, contained more condensed and darker mitochondrial matrix and fewer cristate. The configuration of the internal mitochondrial membrane and the appearance of the matrix indicated the transitional type of mitochondria. The hair cell mitochondria tended to approach the condensed state, while those in the dendritic synapses tended to enter higher levels of the transitional state. Our preliminary report suggests the possibility of the stereologic study on mitochondrial ultrastructure in various states of synaptic function.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (353—356)

SMOOTH ENDOPLASMIC RETICULUM IN HEPATOCYTES OF REGENERATING LIVER — STEREOLOGICAL AND BIOCHEMICAL INVESTIGATIONS

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Smooth endoplasmic reticulum (SER) or hepatocytes plays the important role in numerous metabolic processes such as hydroxylation of toxic apolar substances, biosynthesis of phospholipids and so on. The details of the biogenesis of SER remain obscure. The aim of the present study was the morphological and biochemical evaluation of changes undergoing in SER of hepatocytes during the early phase of liver regeneration. Previously we described corresponding changes of rough endoplasmic reticulum (RER) [1].

Experiments were carried out on Wistar male rats subjected to partial hepatectomy according to Higgins and Anderson [2]. The liver was taken 2, 6, 12, 24 and 72 hours after hepatectomy. Electron microscopy of ultrathin sections with Philips EM 300 device [3], and stereological analysis of SER according to Weibel et al. including the evaluation of volume density (V_v) and surface density (S_v) were performed. The ratio V_v SER/S_v SER being the measure of changes of diameter of SER canaliculi was calculated. Subcellular fractions including that of smooth vesicles (fraction S) were isolated according to our own scheme of differential centrifugation [4]. Protein was assayed with biuret method [5] and activity of glucose-6-phosphate (E.C.3.1.3.9) according to Swanson in our modification [6]. RNA contents [4] and marker enzymes: succinate dehydrogenase (E.C.1.3.9.9.1), 5-nucleotidase (E.C.3.1.3.5), and acid phosphatase (E.C.3.1.3.2.) were assayed as well [7].

Stereology revealed the significant growth (more than 70%) of S_v SER in the earliest time, i.e. 3 hours after hepatectomy (Fig. 1) and even

more pronounced 6 hours later. About the same changes of V_v SER could be seen (Fig. 2). The ratio V_v/S_v did not undergo significant changes for 72 hours of regeneration. Yet, it seemed to be about 25% higher at 72 hour when compared with the control value, what was unsignificant.

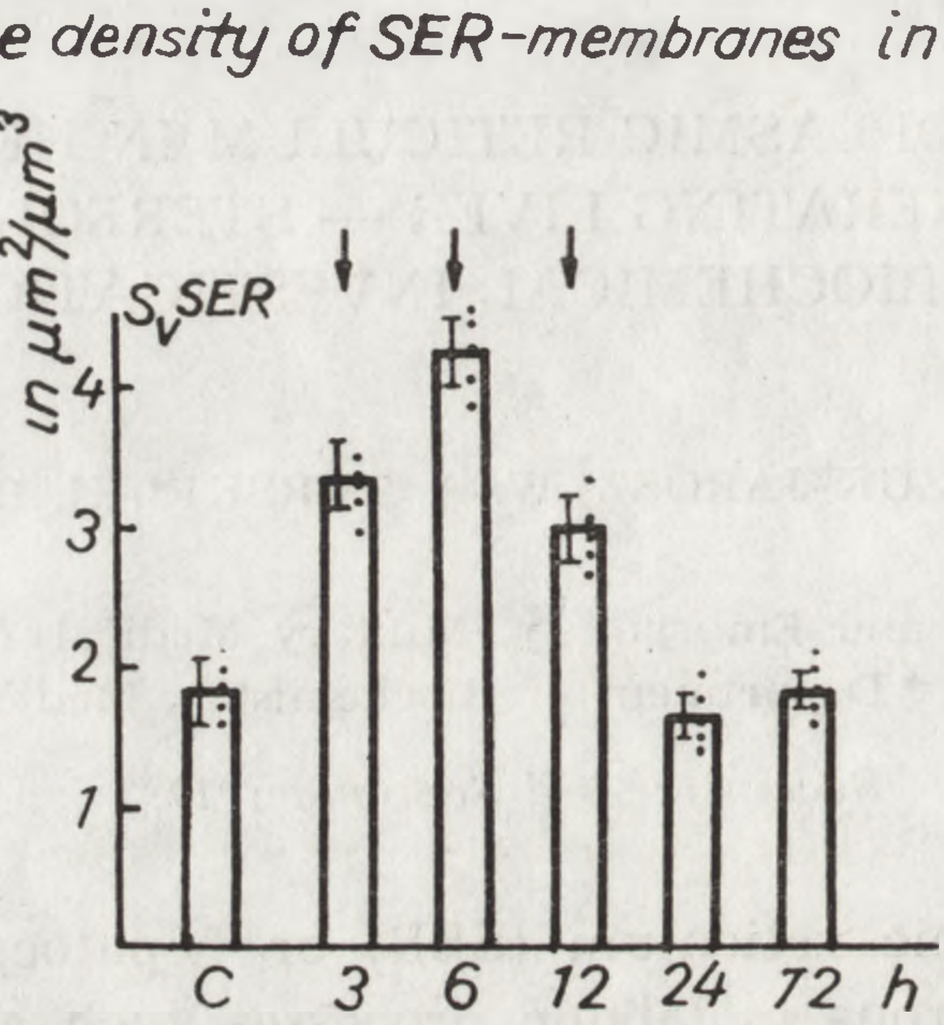

Fig. 1. Surface density of SER (S_v SER) in the control (C) and experimental groups

Protein contents of fraction S (Fig. 3) were significantly (about 60%) higher at 3 hour of regeneration than in control rats. A statistically significant drop of this parameter occurred between 3 and 12 hour. These data confirm stereological results.

However, some discrepancies exist as the biochemically evaluated

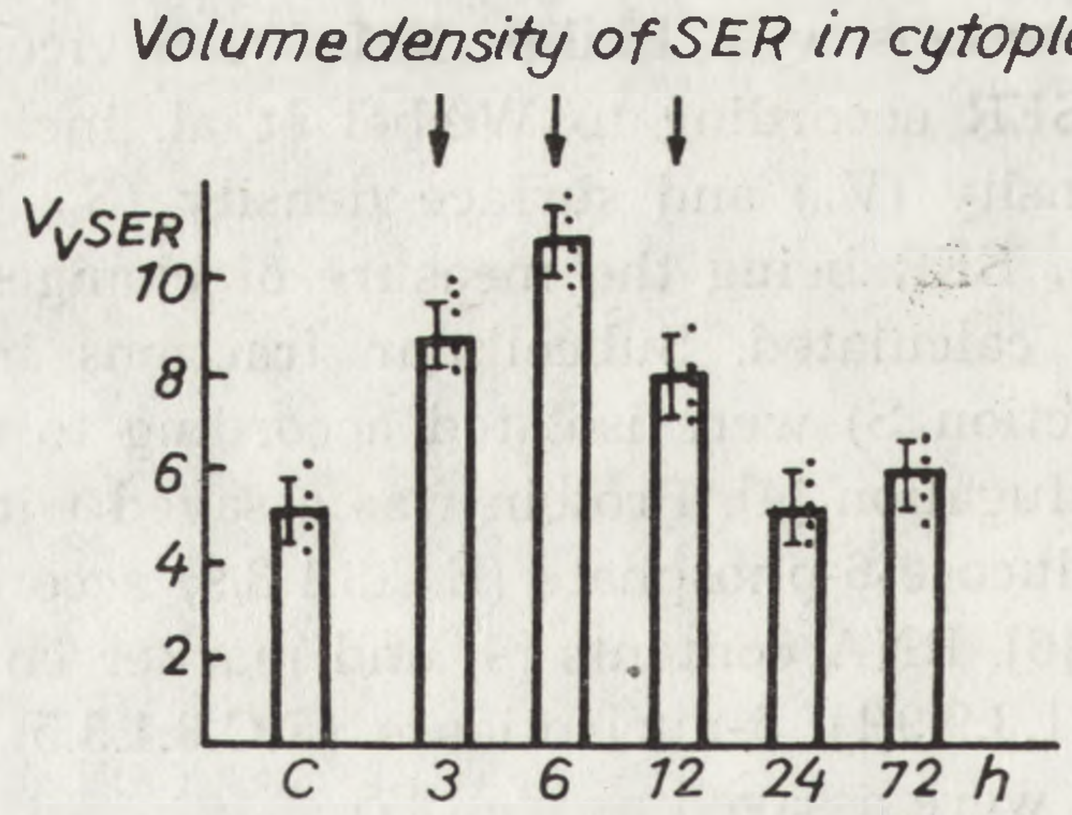

Fig. 2. Volume density of SER (V_v SER) in the control (C) and experimental groups

increase of SER is roughly limited to 3 hours, while the stereological one can be seen at 6 and 12 hour as well. It might be explained with some contamination of fraction S with smooth vesicles coming from Golgi apparatus. The electron microscopy (Fig. 4) of fraction S showed

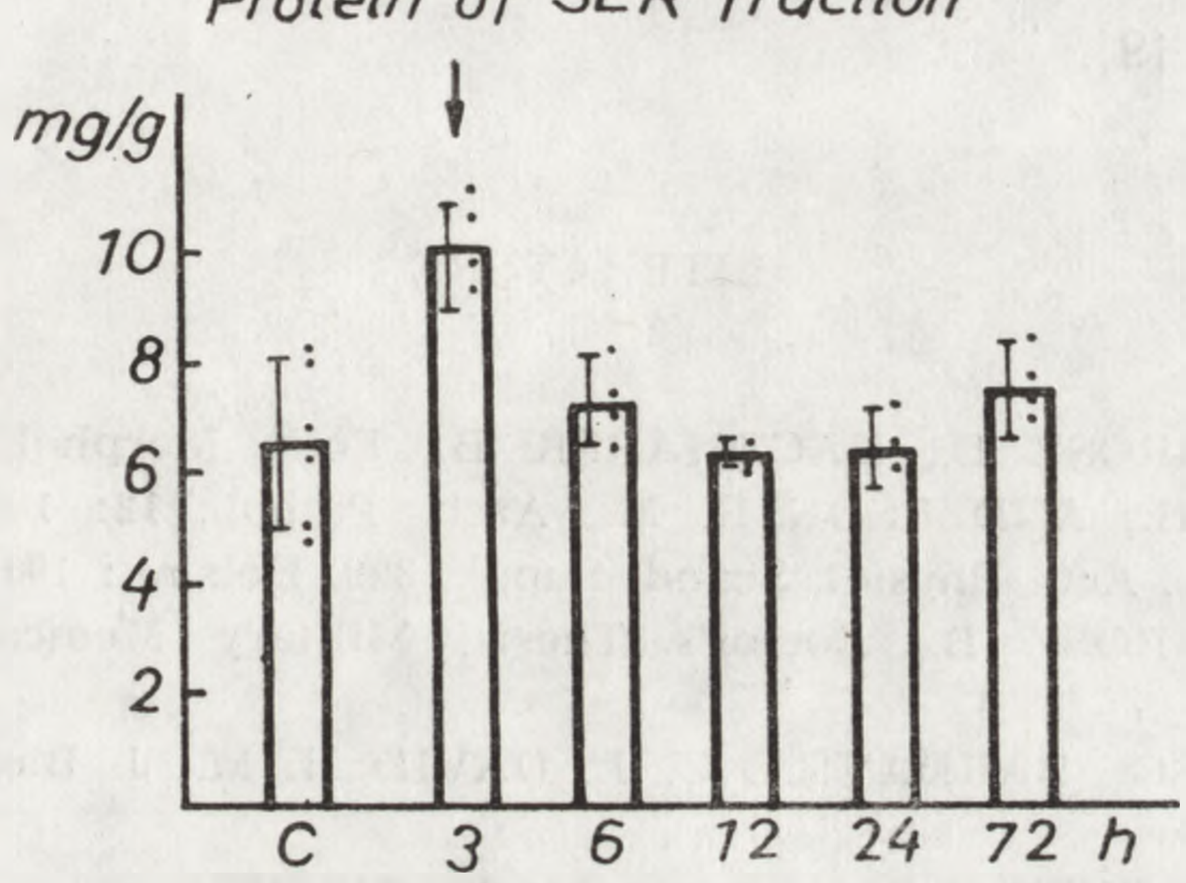

Fig. 3. Protein contents of fraction S in the control (C) and experimental groups

that there were virtually no rough vesicles. Both, small RNA contents and negligible activities of enzymes from other structures confirm purity of the fraction S.

Changes of glucose-6-phosphatase activity in the fraction S did not correlate with that of protein. There was no increase; on the contrary even some small but significant decrease at 6, 24 and 72 hour. One

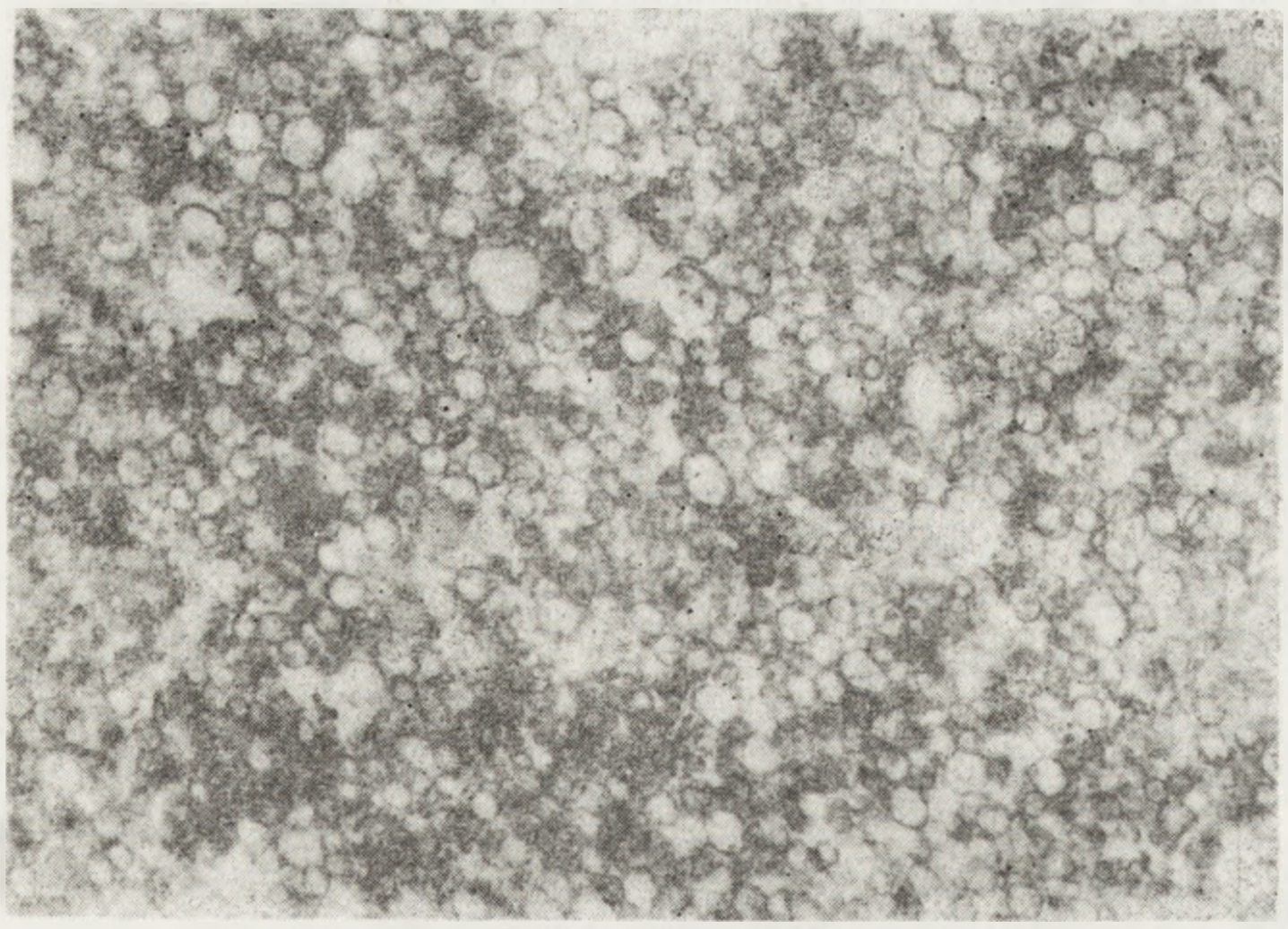

Fig. 4. Ultrastructural picture of fraction S. Magn. × 17 000

might assume that different proteins were built in the "mosaic" structure of SER membranes with different velocity. This would confirm some earlier ideas of Palade [8].

In conclusion one must say that changes in the liver SER growth are somehow oscillatory. After very early growth of SER some drop takes place. This very early growth of SER confirms some of our previous observations [9].

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (357—360)

ACTION POTENTIAL IN *NITELLOPSIS OBTUSA* INDUCED BY UV-RADIATION

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Action potential seems to be the universal phenomenon in biomembranes. It has been extensively investigated in ionic and electrical terms in *Charophyte* cells [1, 3] and recently in *Acetabularia* [2, 4]. However, the molecular mechanism of its generation still remains unclear. One of the approaches to resolve this is the investigation of the action potential in plant cells, induced by UV-radiation. The aim of the present work was to find out whether the changes of electrical potential occurring in response to irradiation of cells of *Nitellopsis* obtusa with UV-light fulfils the criteria of the action potential. The influence of temperature and KCN on UV-induced potential changes was also investigated.

The experiments were performed with cells of *Nitellopsis obtusa* cultured in artificial pond water. During the measurements the cells were kept in solution of the following composition: 0.1 mM KCl, 1.0 mM NaCl, 1.0 mM $CaCl_2$. The membrane potential (i.e. the electric potential difference between the vacuole and external medium) was measured by means of the standard microelectrode technique. The high-pressure mercury vapour lamp was used as the source of UV-radiation.

Triggering of action potential by UV-radiation. The results obtained in this work showed that UV-radiation caused depolarization of membrane potential. The amplitude of depolarization being dependent on the duration and intensity of UV-irradiation. If the UV-exposition exceeds a threshold level the action potential was observed, as illustrated by the results of the experiment shown in Fig. 1. Propagation of the action potential. Action potential initiated in one cell was conducted across a node to the adjacent cell (Fig. 2). Spontaneous action potential. In many cases, single or repetitive action potentials occurred spontaneously (Fig. 3). This phenomenon clearly shows that it is not the

energy supplied by the stimulation process which drives action potential, but it must be fuelled by endogenous energy source. We have also found that such factors as temperature and KCN change the shape of action potential (Fig. 4). Influence of temperature and KCN on

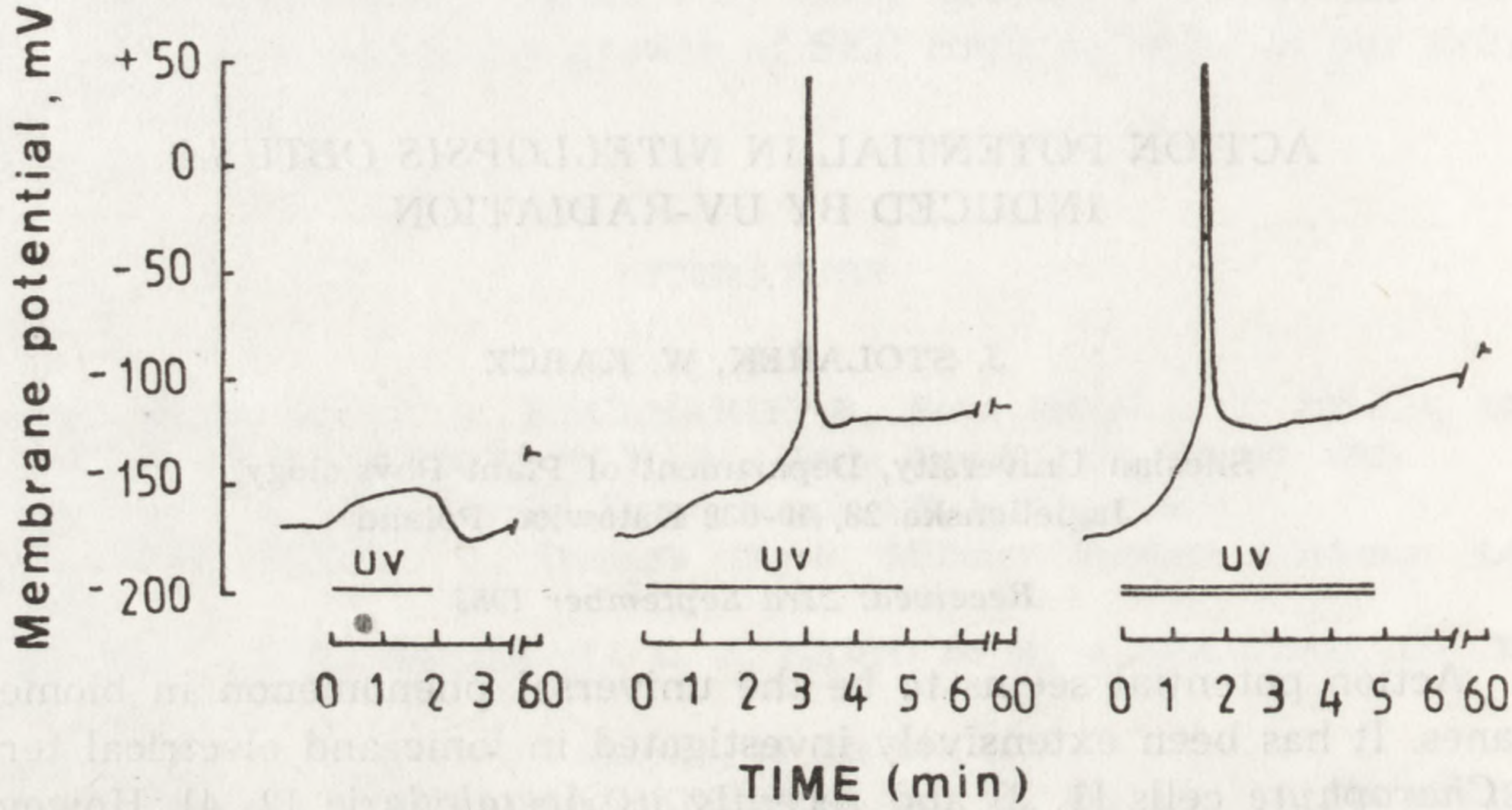

Fig. 1. Example of the response of the membrane potential of *Nitellopsis obtusa* upon UV-radiation of different duration and intensity

the action potential induced by UV-radiation observed by us supports the view-point according to which active ion transport maintained by some metabolic processes might be involved in the generation of action potential induced by UV-irradiation in plant cells.

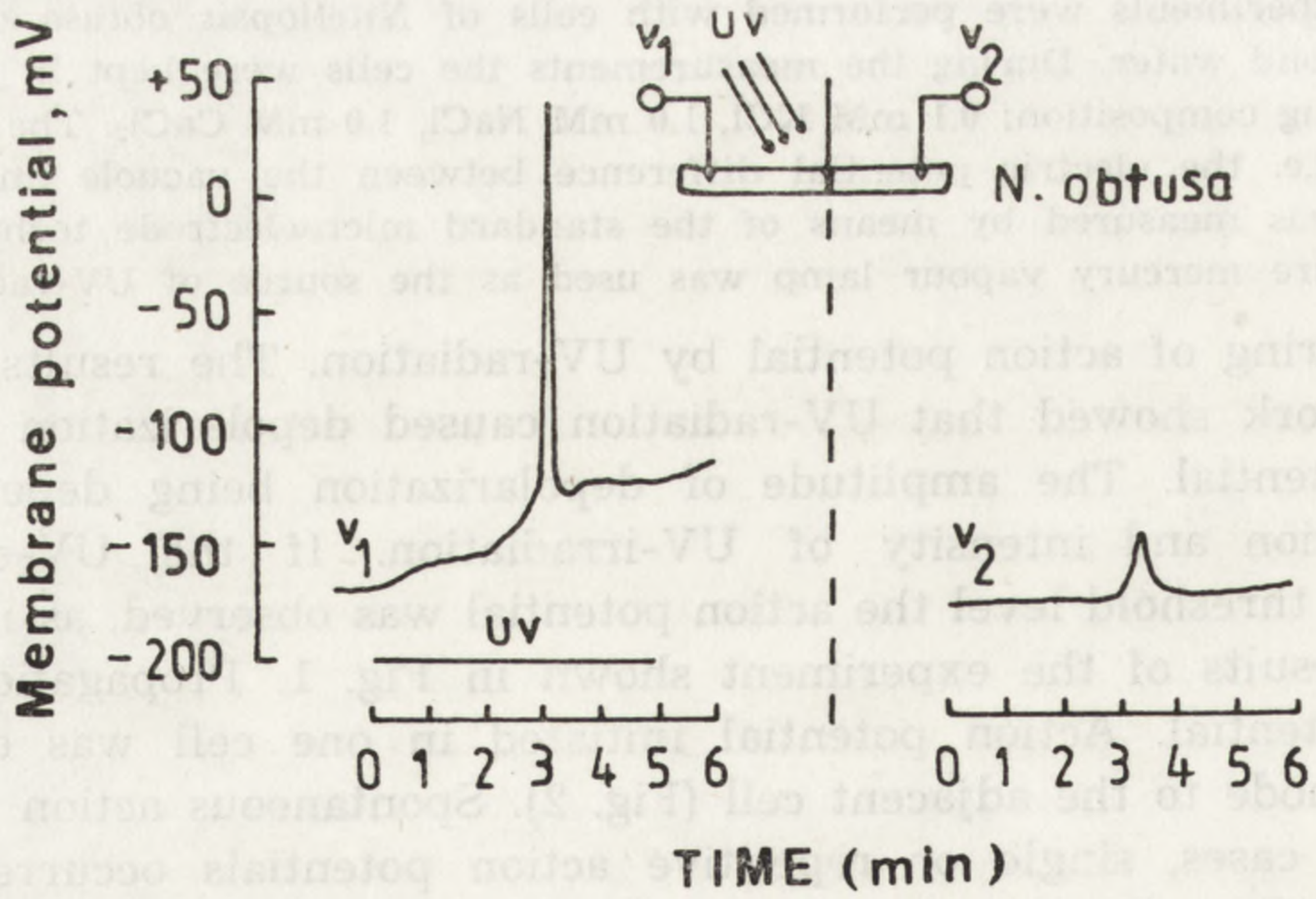

Fig. 2. Propagation of the action potential induced by UV-radiation

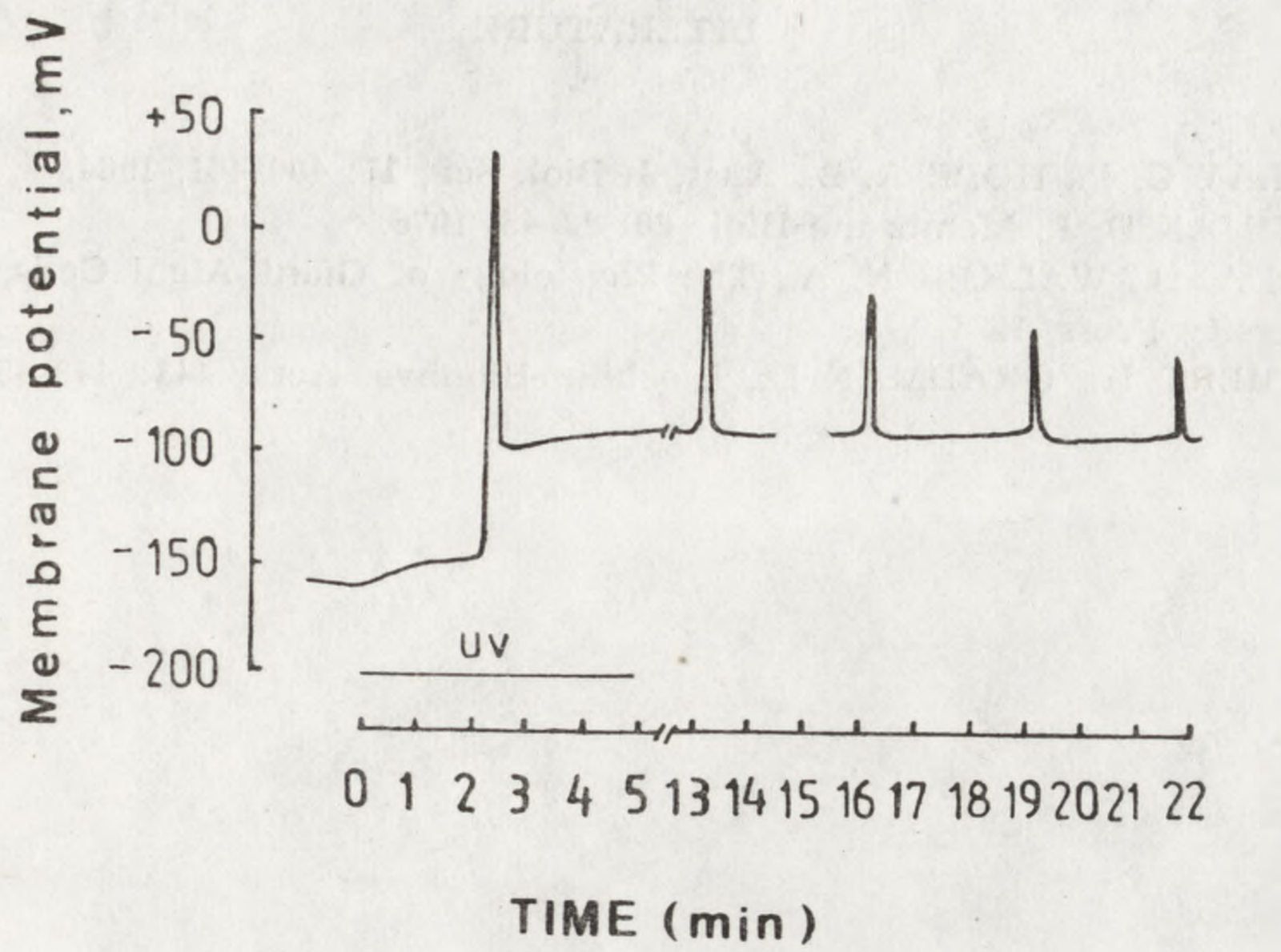

Fig. 3. Spontaneous action potentials

It follows from the presented data that UV-induced electrical potential differences occurring in Nitellopsis obtusa fulfil the criteria of the action potential, that is a transient depolarization with all-or-none characteristics (Fig. 1), endogenous energy supply (Fig. 3) and propagation (Fig. 2).

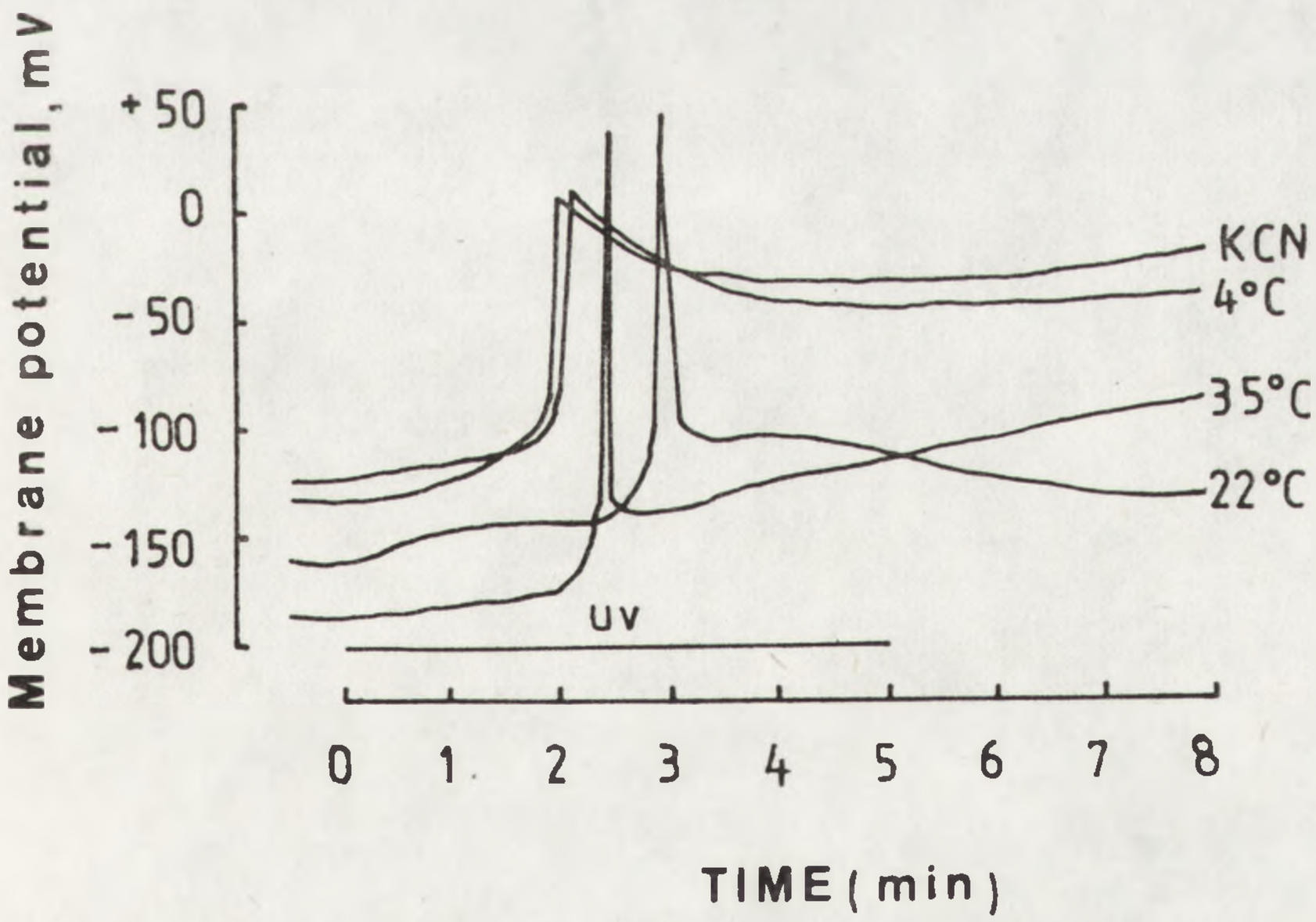

Fig. 4. Effect of temperature and KCN on the action potential induced by UV--radiation

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (361—364)

THE ACTION OF PHYTOHORMONES ON RESTING AND ACTION POTENTIAL IN HIGHER PLANTS

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Several investigators have found that permeability of the protoplasmic membranes and induction of electric potential differences in plant cells is affected by the action of auxin (IAA) on the plasmalemma [1, 2, 3, 7]. However, there are no reports in literature on the relation between the action of auxins and other phytohormones on the excitation processes in higher plants. In the paper we present data on the influence of IAA, gibberellin (GA_3) and kinetin on membrane potentials on cells of coleoptile of *Triticum vulgare* L. and on the amplitude and propagation of action potentials induced in *Phaseolus vulgaris* L.

Measurements of plant electrical potentials were performed with a standard electrophysiological technique [6, 8, 9]. In our investigations intracellular microelectrodes and surface electrodes were used (Fig. 1). Hormones were added to the bathing media of the concentration of 10^{-5} M in the case of membrane potential measurement and to the nutrient solution of the concentration of 10^{-4} M in experiments with action potentials. The action potentials were induced by a 3 min flash of white light of the intensity of 1008 W/m². In another series of experiments action potentials were induced with thermal stimulation.

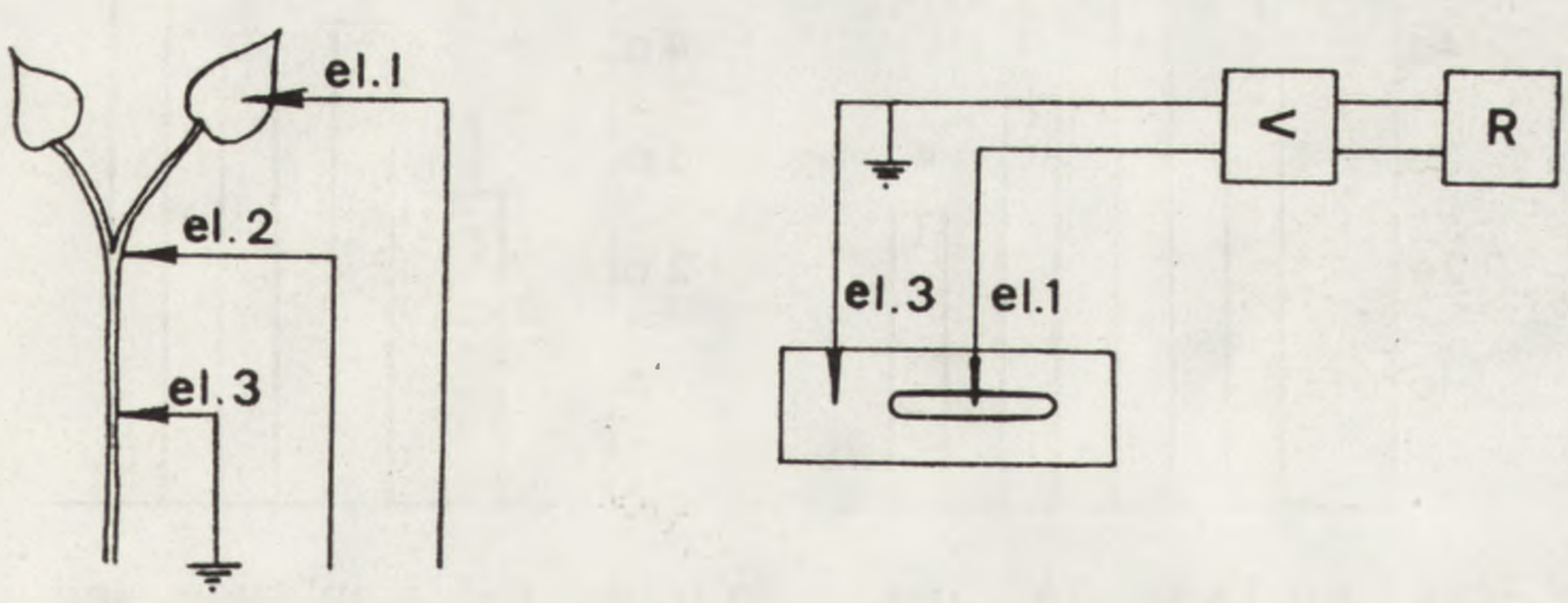

Fig. 1. Block-diagram of apparatus; el 1,2 recording electrode, el 3 reference electrode, R recorder

Data presented in Fig. 4 show, that incubation of the wheat coleoptiles in the media containing auxin or gibberellin induced hyperpolarization of the cell membrane potential, whereas kinetin had no effect. On the other hand, the amplitude of the action potentials in *Phaseolus vulgaris* L. (induced both by thermal and light stimulation) was changed

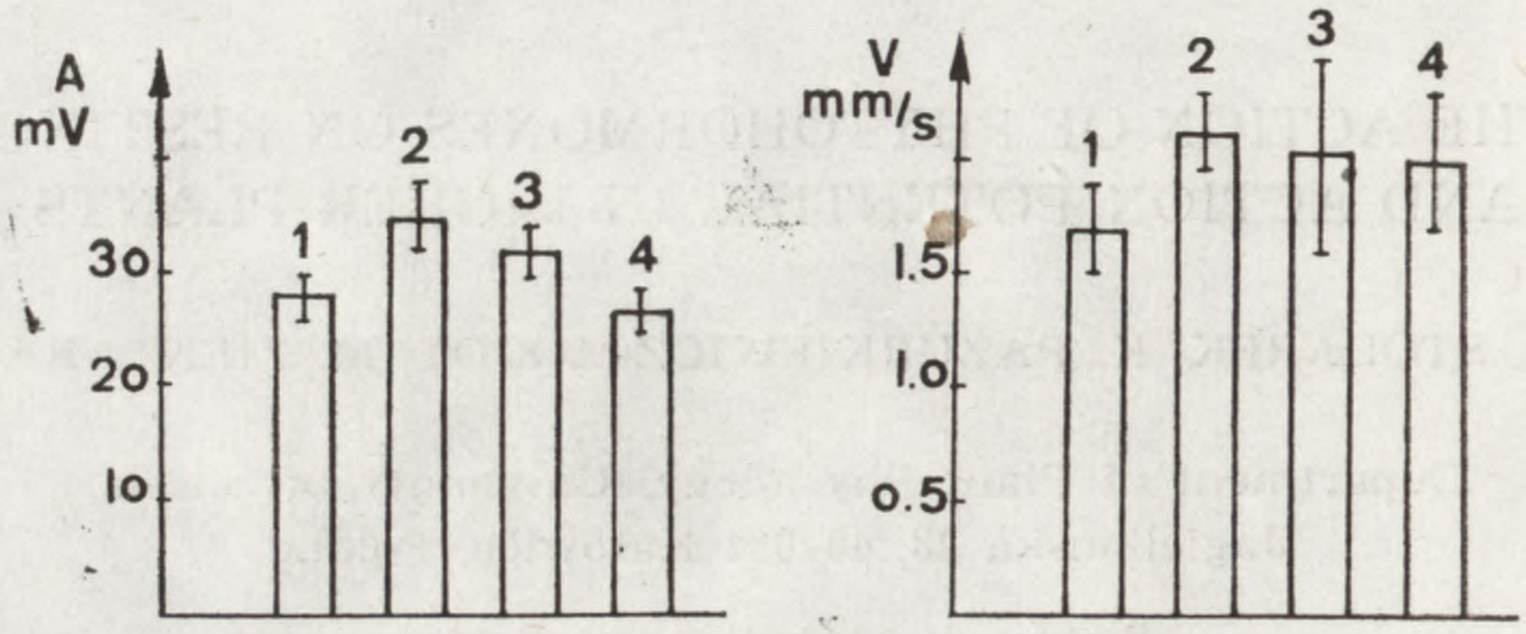

Fig. 2. Amplitude and velocity of the conduction of action potentials induced by thermal stimulation in plants treated with IAA, GA_3 and kinetin; *1* — control, *2* — IAA, *3* — GA_3, *4* — kinetin

only in plants grown in the nutrient solution with addition of auxin, whereas the propagation of the action potential was significantly stimulated by all investigated phytohormones (Figs. 2, 3, 5).

Considering our data and taking into account the fact, that auxin affects the permeability and electrical properties of membranes [4, 5, 7] one can assume that the mechanism of action of other phytohormones at membrane level is similar. Thus, phytohormones control the permeability and electrical properties of membranes and, in consequence, modify the response of plants to envirommental factors.

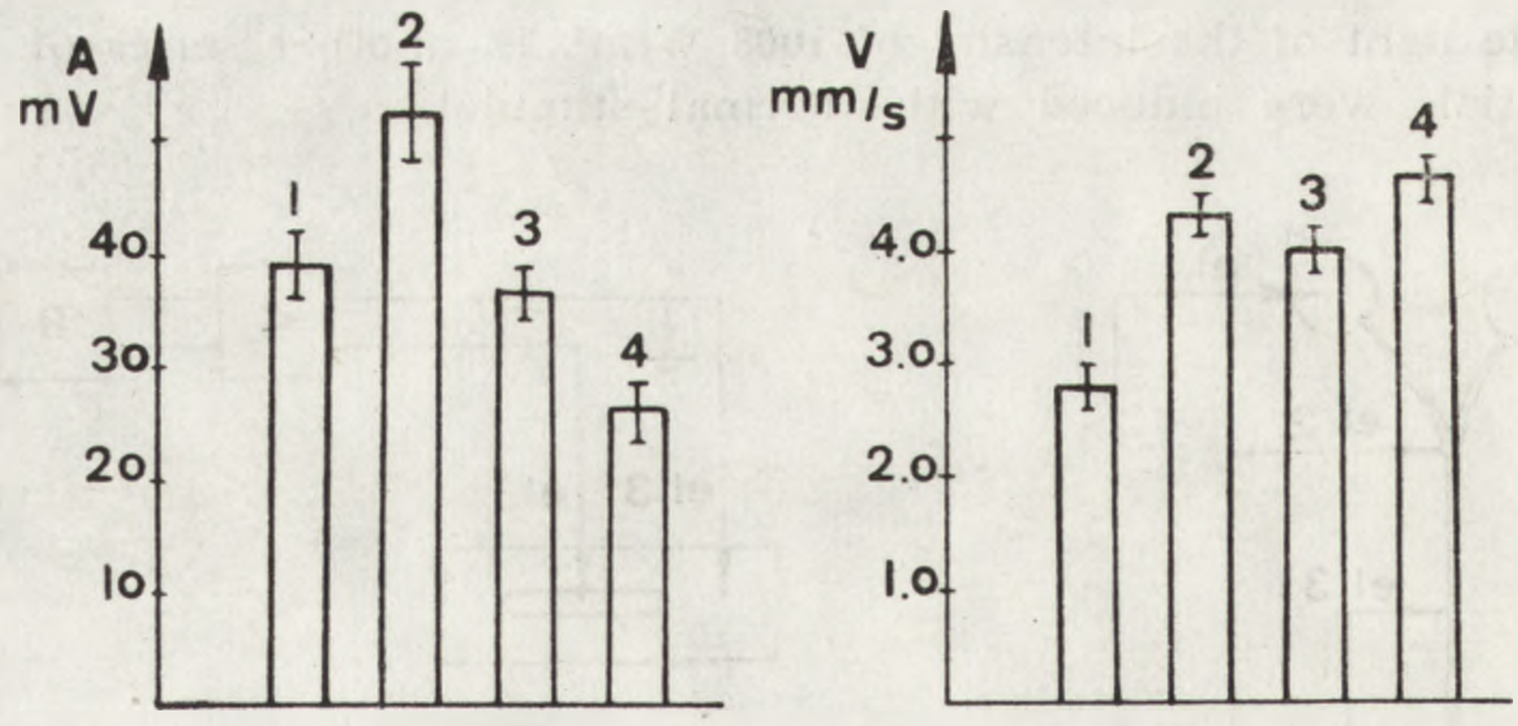

Fig. 3. The effect of IAA, GA_3 and kinetin on the amplitude and conduction velocity of action potentials induced by light in higher plants; *1* — control, *2* — IAA, *3* — GA_3, *4* — kinetin

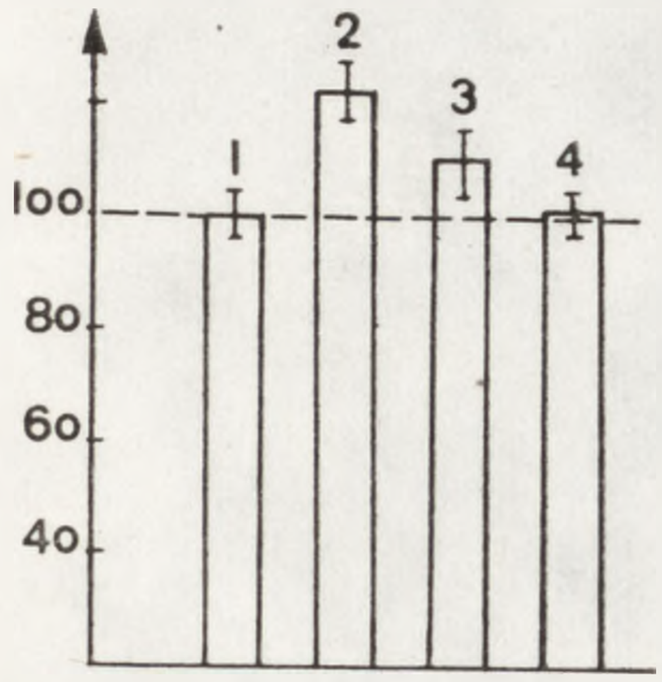

Fig. 4. The effect of IAA, gibberellin and kinetin on membrane potential of wheat coleoptile cells; *1* — control, *2* — IAA, *3* — GA_3, *4* — kinetin

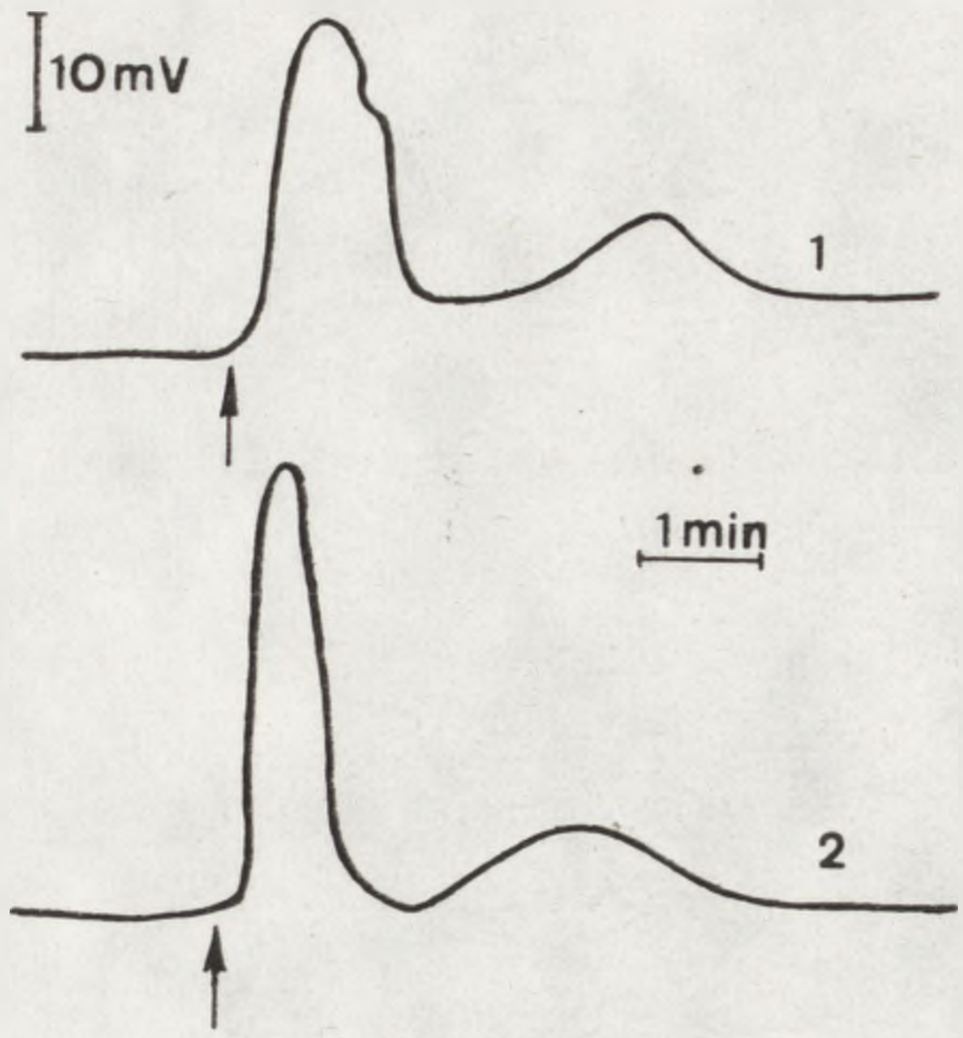

Fig. 5. Action potentials induced with thermal stimulation in plants

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (365—368)

LIGHT-INDUCED ELECTRIC REACTION OF PLANTS WITH C_3 AND C_4 PHOTOSYNTHETIC PATHWAYS

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The effect of light on electrical properties of plant cells was studied by various authors [1, 3, 4, 6, 7]. However, the data available from the literature concern mainly light-promoted changes of transmembrane electric potential differences in plasmalemma of giant algal or aquatic moss cells, whereas only few papers deal with photoelectric response of higher plants [5]. The mechanism of light-driven action potential in higher plants is so far unknown, although there is some evidence that both photosynthetic [2, 4] and phytochrome pigment systems are involved in light-induced electrogenesis [4].

The purpose of the present work was to compare photoelectric response of plants with C_3 (*Phaseolus vulgaris* L. var. wiejska, *Cucurbita pepo* L. var. złota) with plants possessing C_4 photosynthetic pathway (*Zea mays* L.).

The experiments were carried out with 15 days old plants grown in standard media and controlled light and temperature conditions. Action potentials were triggered by white light flashes of appropriate duration. The intensity of the light as measured with a Kipp and Zonnen thermopile (CA-2) at the leaf level was 75 $W \cdot m^{-2}$. The arrangement of the recording calomel surface-contact electrodes in plants is shown in Fig. *1a,b,c*. The electrodes were joined via high-input impedance amplifiers to a six-channel high-speed chart recorder (Rika-denki KB-66, Japan).

Prior to electrophysiological experiments plants had been maintained in darkness for 40 min. This pretreatment was found to be the essential requirement in order to make the plant ready for light excitation.

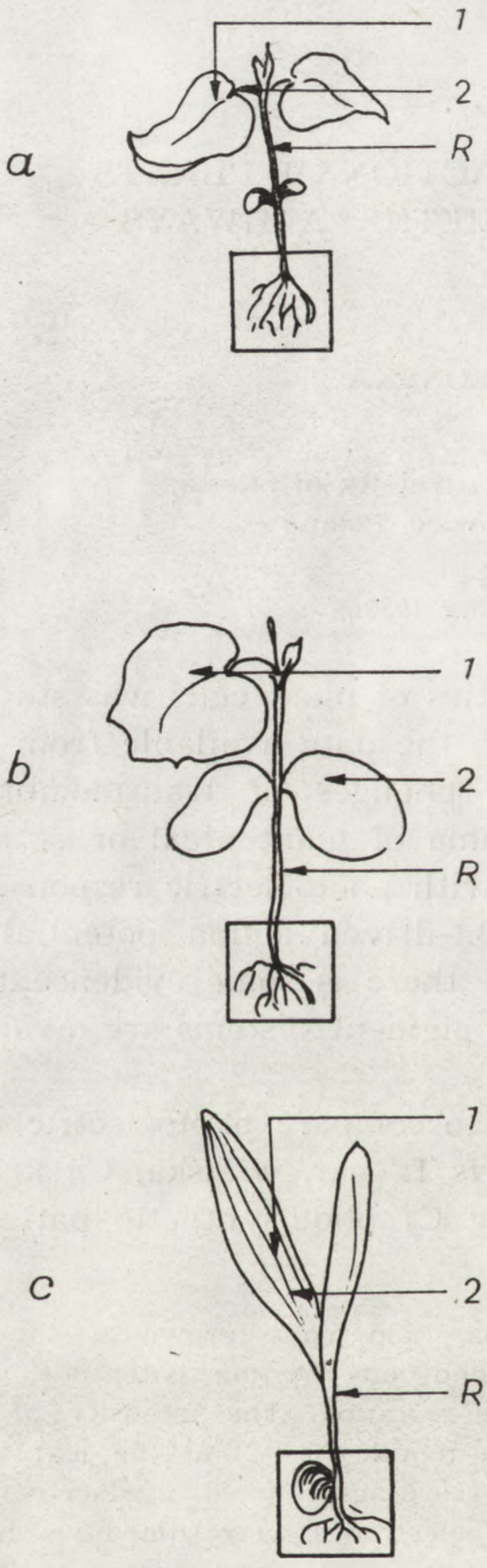

Fig. 1. The arrangement of recording electrodes on investigated plants; *a* — *Phaseolus vulgaris* L., *b* — *Cucurbita pepo* L., *c* — *Zea mays* L.; *1,2* — recording electrodes, R — reference electrode

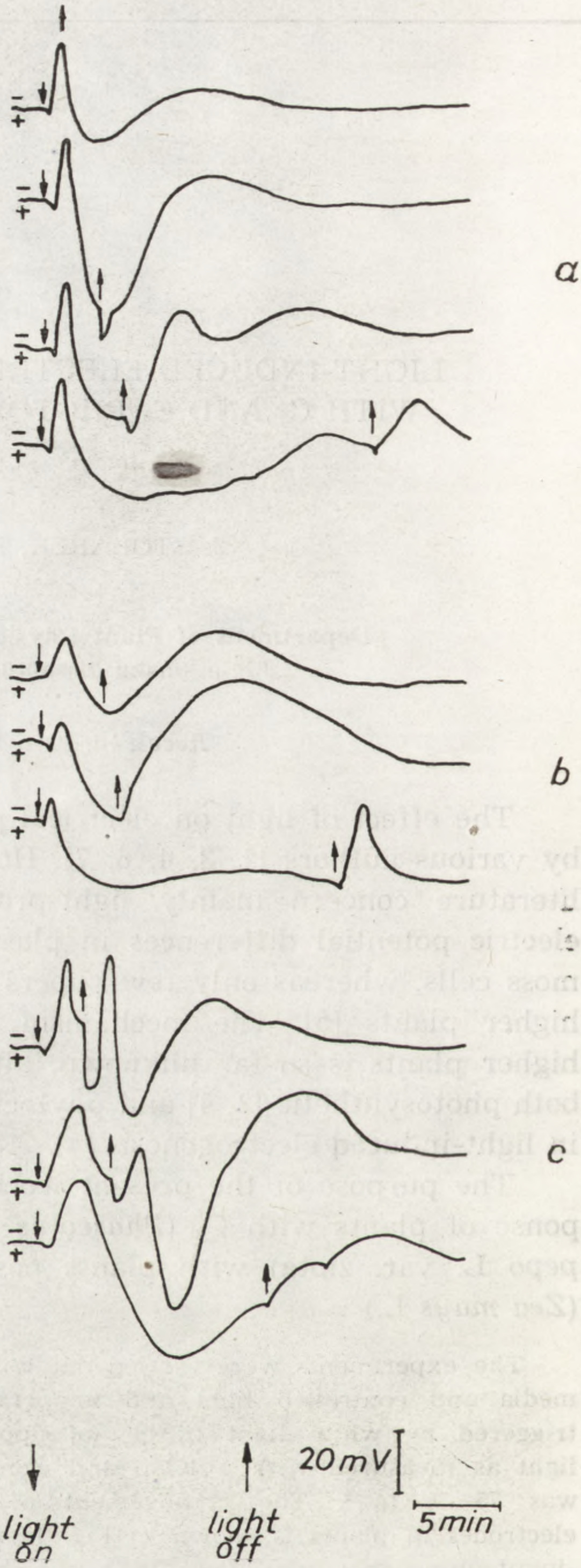

Fig. 2. Typical examples of electric potential changes induced by light switching on and off in three investigated species; *a* — *Phaseolus vulgaris* L., *b* — *Cucurbita pepo* L., *c* — *Zea mays* L.

Typical examples of electric potential changes induced by light switching on and off, recorded in bean, pumpkin and corn plants are shown in Fig. *2a*, *b*, *c*.

We have found that photoelectric response of all investigated plants resulted in appearing of an action potential of a diifferent shape in plants with C_3 and C_4 photosynthetic pathways Fig. *2a*, *b*, *c*.

Substantial differences were found in the response of the two types of plants to light switching off. In the case of corn a fast hyperpolarization was observed in contrast to two other species (pumpkin and bean), where a large depolarization took place Fig. *2a*, *b*.

Although further research is needed in order to elucidate the nature of photoelectric response of both types of plants, our results indicate that the electrophysiological reaction of higher plants to light is closely related not only to the operation of photosynthetic pigment systems but also to the entire photosynthetic process, including its dark phase.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (369—372)

ENKEPHALIN INACTIVATION IN BRAIN SYNAPTIC ENDINGS: REUPTAKE OR EXTRACELLULAR DEGRADATION

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Since enkephalins, endogenous opioid peptides, are considered to be putative neurotransmitters modulating perception of pain, they should fulfil the criterion of being rapidly eliminated from the synaptic cleft. The present studies evaluate the role of the two possible mechanisms of enkephalim inactivation: reuptake and extracellular degradation. The uptake and degradation of triatiated Leu-enkephalin, D-Ala^2-Leu-enkephalin amide and D-Ala^2-D-Leu-enkephalin were followed using cell fractions enriched in astrocytes, synaptosomes and 138 MG glioma, C6 rat glioma and N_{18} mouse neuroblastoma cell lines. The cells and synaptosomes were incubated for 10 to 30 min at 0°C, 30°C and 37°C in Krebs-Ringer solution containing radioactive enkephalins. The radioactive products in the tissue and in the medium were identified chromatographically on Sephadex LH 20. It was demonstrated that upon incubation with cells and synaptosomes ^{3}H-Leu-enkephalin is rapidly degraded with the participation of aminopeptidase (s) releasing ^{3}H-Tyr and carboxypeptidase(s) liberating ^{3}H-Tyr-Gly-Gly. The contribution of carboxypeptidase(s) to the total decomposition of the peptide in glioma 138 MG was 20% at 0.7×10^{-8} M enkephalim and 10% at 4.6×10^{-6} M enkephalin concentrations, whereas in N_{18} neuroblastoma cells the carboxypeptidase was responsible for 36% of the degradation at 4.5×10^{-6} M enkephalin. In synaptosomes the degradation of 10^{-5} M enkephalin was dependent on carboxypeptidase activity in 47%. These results may be attributed to higher activity of enkephalinase, specifically releasing Tyr-Gly-Gly from enkephalins, in neuronal and

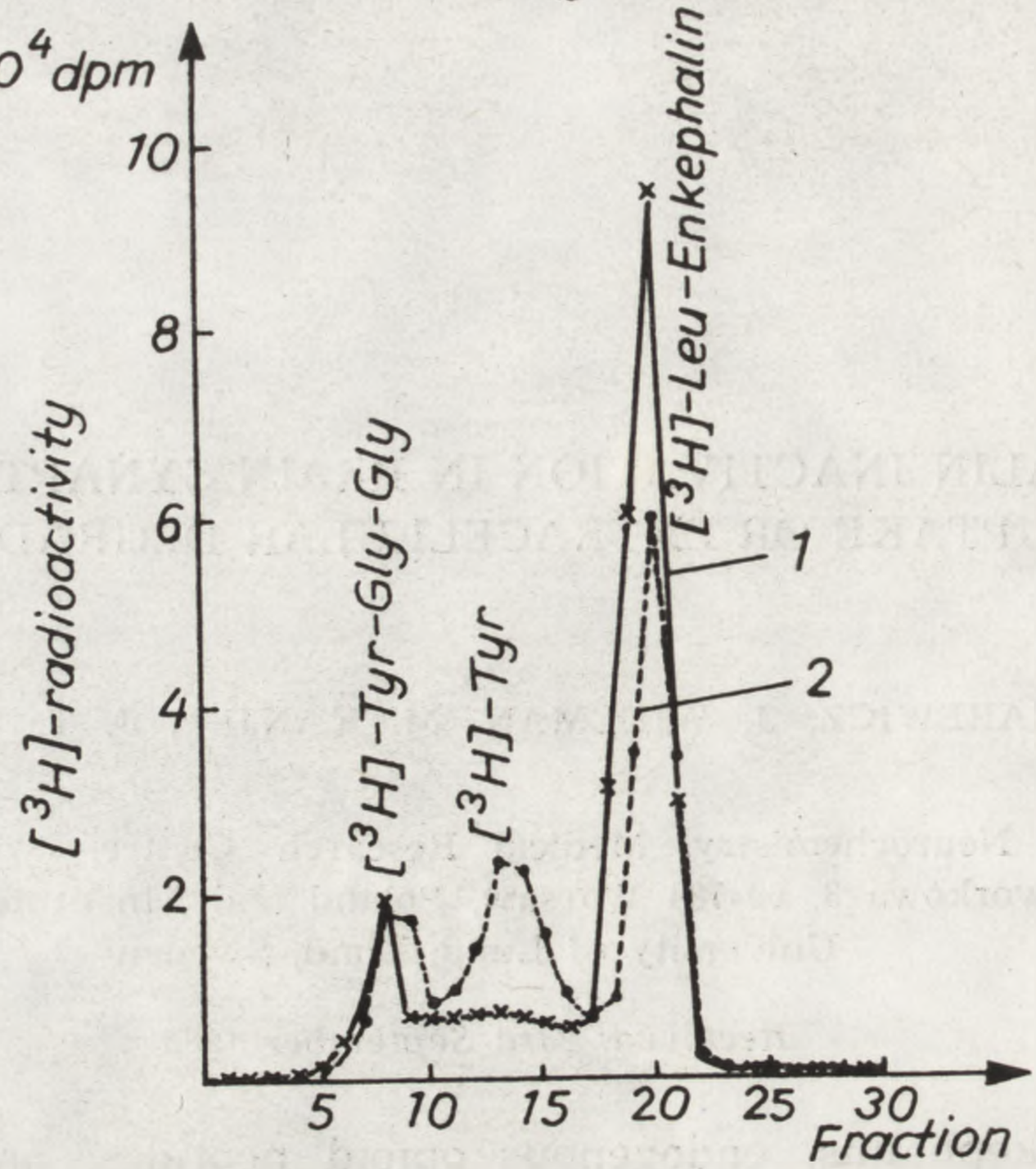

Fig. 1. ^{3}H-Leu-enkephalin degradation by 138 MG glioma cells; *1* — 0 min incubation, *2* — 10 min incubation

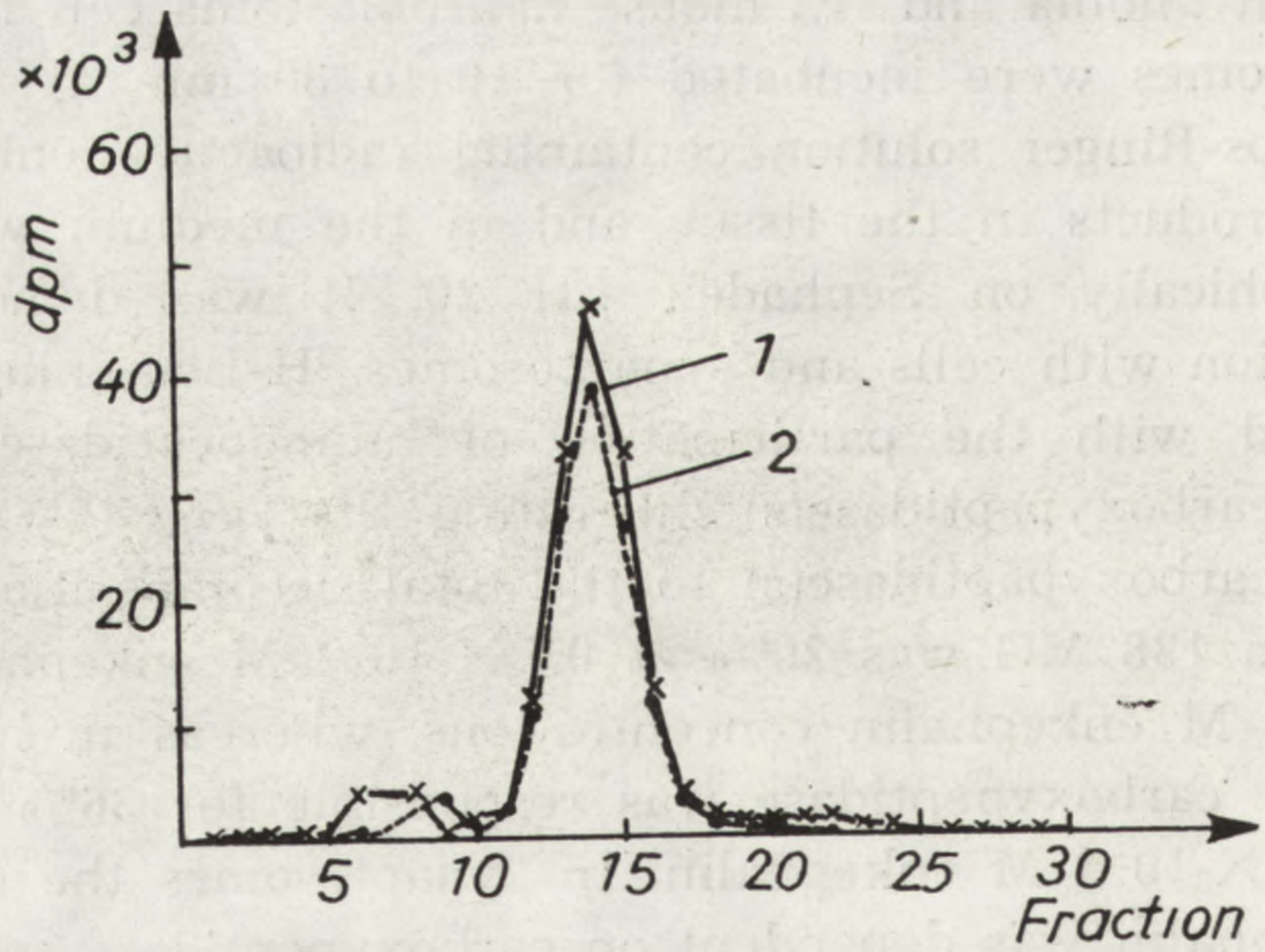

Fig. 2. Sephadex LH 20 fractionation of an extract from 138 MG cells incubated with ^{3}H-Leu-enkephalin; *1* — [^{14}C]-tyrosine, *2* — cell extract

particularly in synaptosomal membranes. It was also demonstrated that 1 mM o-phenantroline completely inhibited enkephalin degradation. The resistance of D-Ala^2-Leu-enkephalin amide to the enzymatic degradation was also confirmed. The radioactivity accumulated in brain cells and synaptosomes during the incubation with ^{3}H-Leu-enkephalin, as analysed chromatographically, was found to be predominantly ^{3}H-Tyr. This accumulated tyrosine could have been either derived from the pool liberated extracellularly, prior to tyrosine uptake, or be released intra-

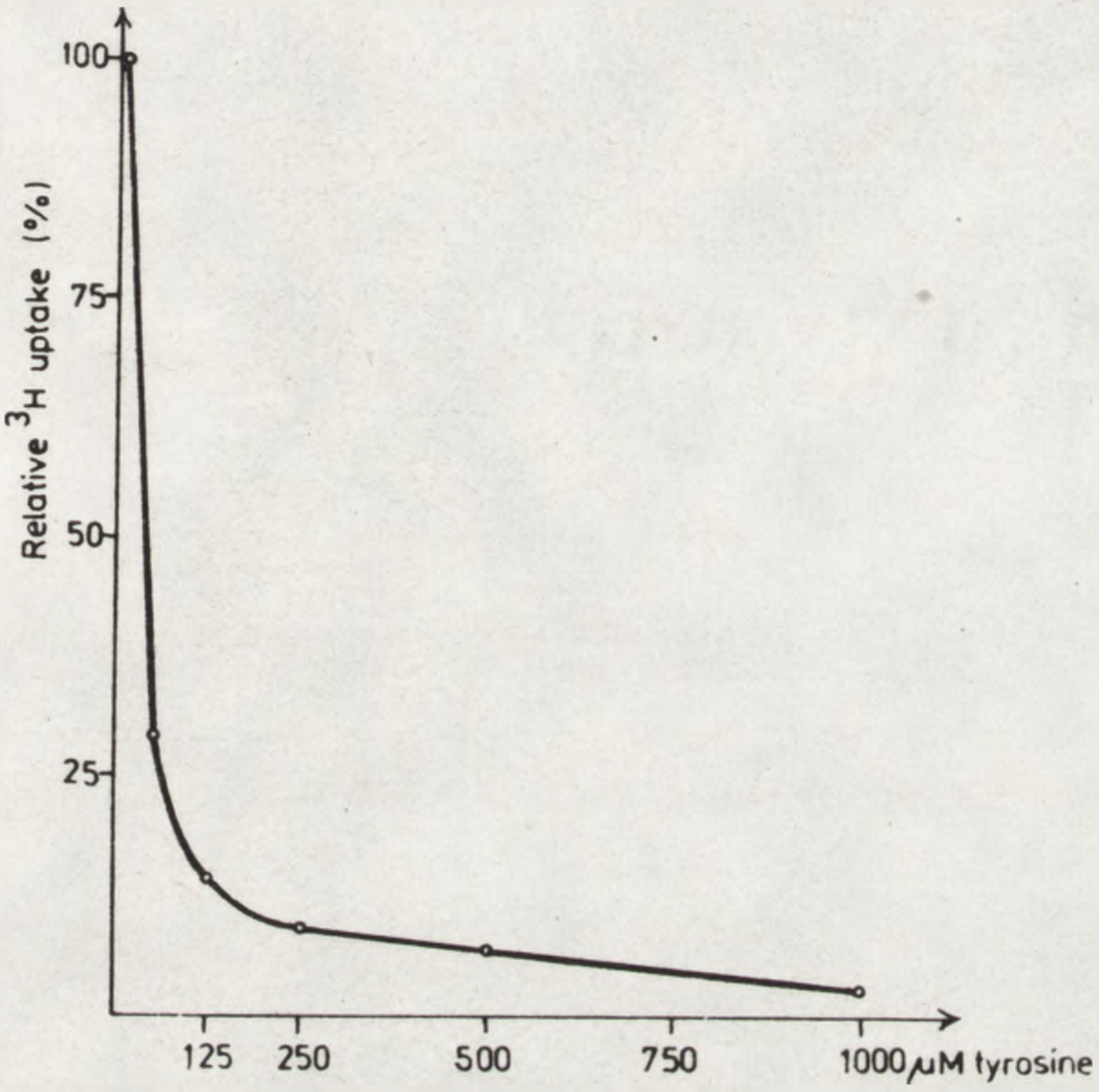

Fig. 3. Effect of tyrosine on ^{3}H uptake by 138 MG glioma cells incubated with ^{3}H-Leu-enkephalin

cellularly from the already accumulated enkephalin. It was found that the cells and synaptosomes used in these studies accumulate tyrosine by means of the high affinity uptake system. Addition of 1 mM cold tyrosine to minimize the interference of free ^{3}H-Tyr liberated extracellularly, or the use of 1 mM o-phenantroline to inhibit enkephalin degradation, dramatically decreased the rate of ^{3}H-tyrosine accumulation in the cells and cell fractions studied. The inhibition was more than 90% in experiments with cultured cells and 80% in synaptosomes. In experiments with isolated astroglia 1 mM tyrosine inhibited ^{3}H-Tyr accumulation by 56%, whereas o-phenantroline was effective in 87%. It was also found that D-Ala^2-Leu-enkephalin amide and D-Ala^2-D-Leu-enkephalin, both peptides resistant to degradation, were not accumulated by cultured cells and synaptosomes. The results show the inability of

cultured and isolated brain cells and synaptosomes to accumulate enkephalins and demonstrate the activity of degradative enzymes in there. Since preliminary experiments failed to demonstrate the release of detectable amounts of peptidases to the growth medium even after 3 days of cell culturing, decomposition of enkephalin should rather be ascribed to the degradative enzymes of plasma membranes oriented towards the extracellular compartment. These results suggest that the extracellular degradation of enkephalins, but not their reuptake, may represent the major mechanism of their inactivation in the CNS.

POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (373—376)

CALCIUM TRANSPORT AND NEUROTRANSMITTER UPTAKE IN RAT BRAIN SYNAPTOSOMES MODIFIED BY EXOGENOUS GANGLIOSIDE AND NEURAMINIDASE

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Synaptosomes, the nerve endings fraction of brain homogenate, are particulary rich in gangliosides, suggesting specificity of their function.

As shown recently, exogenous GM_1 interact and become associated with crude neuronal membranes, affecting some enzyme activities [1]. In the present studies GM_1 was applied in 40 nM and 400 nM concentration being relevant to the appearance of two different physical forms of ganglioside dispersion. According to Toffano et al. [1] each of them exhibit different mechanisms of interaction with the membrane.

The binding of GM_1 in 40 nM concentration to the synaptosomal fraction was measured. It was found that the high affinity binding of GM_1 was augmented by increasing the incubation period to 120 min. A small binding of GM_1 occurred also at 0°C but did not increase with time. Endogenous generation of GM_1 in the synaptoplasmatic membranes

TABLE 1

Effect of specific interaction of GM_1 with plasma membrane on $^{45}Ca^{2+}$ transport by synaptosomes

Conditions	$^{45}Ca^{2+}$ nmol/mg prot./5 min		
	5 mM K^+	50 mM K^+	ΔK
Control	8.34±0.32	29.28±3.40	20.96±4.58
×GM_1 40 nM	9.05±0.17	59.75±1.83	50.70±1.89

Synaptosomes (1 mg/ml) were preincubated in Krebs-Ringer medium, pH 7.4 at 30°C by 60 min with 40 nM GM_1. After centrifugation the synaptosomes were resuspended and the $^{45}Ca^{2+}$ accumulation was started by adding 1.2 mM $^{45}Ca^{2+}$/0.2 μCi/μmol. Incubation was terminated after 5 min by 10 mM EGTA. Means from 4 experiments ± SEM [2].

was achieved by N-ase treatment. Neuraminidase (from Vibrio Cholerae 8 units/ml for 30 min) decreases the total synaptosomal ganglioside content to 77% of control on the account of polysialogangliosides with the concomitant increase (from 13% to 30%) of GM_1 ganglioside.

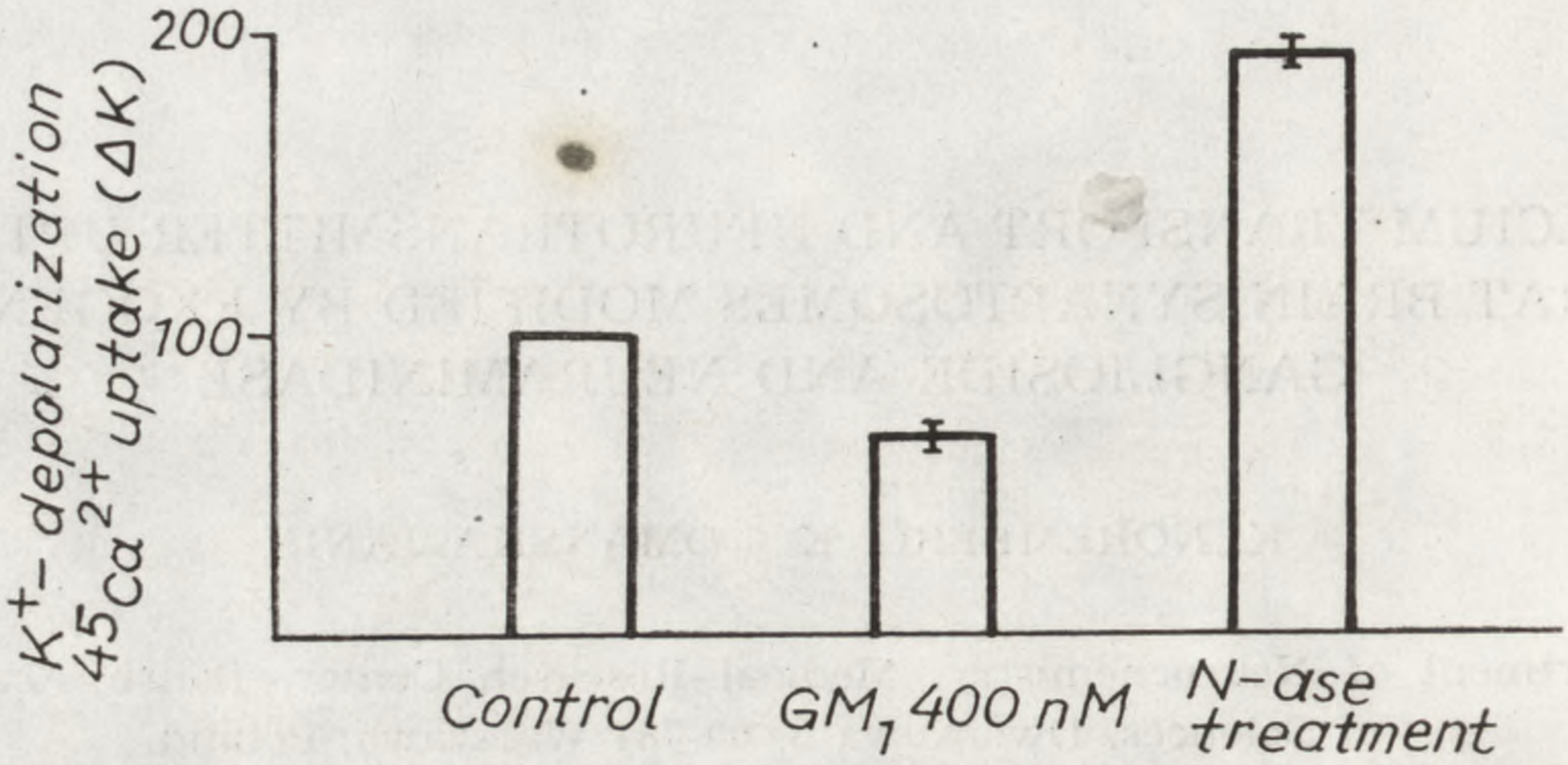

Fig. 1. Effect of higher concentration (micellar) and endogenous generation of GM_1 (N-ase treatment) on K^+-dependent calcium uptake by synaptosomes. Conditions of incubation as indicated in Table 1

In this study calcium transport in control (5 mM KCl) and depolarizing (50 mM KCl) medium in rat brain synaptosomes was investigated. The differences in $^{45}Ca^{2+}$ influx in high and low potassium medium represent the specific depolarization dependent Ca accumulation (K).

TABLE 2

Effect of GM_1 on kinetic constants for the uptake of dopamine, GABA and serotonine by ra brain synaptosomes

Conditions	^{3}[H] – DA		[^{3}H] – GABA		[^{3}H] – 5-HT	
	K_m (nM)	V_{max} pmol/mg prot/min	K_m (nM)	V_{max} pmol/mg prot/min	K_m (nM)	V_{max} pmol/mg prot/min
Control	0.31	23.0	5.0	133.0	0.13	9.52
+GM_1 40 nM	0.31	23.0	5.0	109.0	0.13	14.30
400 nM	0.31	19.2	5.0	109.0	0.59	28.60

Rat brain synaptosomes were preincubated as stated above (Table 1). The uptake of [^{3}H] – DA, [^{3}H] – GABA and [^{3}H] – 5-HT was measured in their different concentrations by 2.5 min in 30°C. Results were calculated from 3-4 experiments.

Specific interaction of GM_1 with synaptoplasmatic membrane as observed in low (20 nM) concentration resulted in marked stimulation of depolarization-induced calcium transport (Table 1). This may be explained by the activation of voltage-sensitive calcium channels.

This effect was reversed by elevation of GM_1 up to 400 nM which produced the partial inhibition of K^+-dependent Ca^{2+} transport (Fig. 1). We also observed that endogenous generation of GM_1 produced by N-ase treatment was associated with activation of depolarization-dependent Ca uptake but also with a marked increase in the passive permeability of the synaptosomal membranes to Ca^{2+}. This finding suggests that N-ase action in vitro can not be exclusively attributed to accumulation of GM_1, but also to unspecific influence on membrane structure.

The uptake of dopamine (DA), serotonine (5-HT) and GABA was studied at various neurotransmitter concentrations. The kinetic constants were calculated using double reciprocal plot analysis. Table 2 summarizes all the results. It can be seen that ^3H—DA uptake system is unaffected to preincubation of synaptosomes with gangliosides. The ^3H—GABA uptake system reacts to both concentration of GM_1 by lowering the V_{max} value from 133 to 109 pmol/mg prot./min. It produces 20% reduction of GABA accumulation in rat brain synaptosomes. The system responsible for the high affinity uptake of ^3H—5-HT exhibits different sensitivities to both examined GM_1 concentration. In low (40 nM) concentration of GM_1 the value of K_m was not changed but V_{max} increased to 14.3 pmol/mg prot./min, i.e. by about 50% as compared with the control value. Consequently, the accumulation of 5-HT in their saturated concentration was found to be significantly increased in synaptosomes. The micellar (400 nM) concentration of GM_1 distinctly alters both K_m and V_{max} values of 5-HT uptake system. However, in saturated concentration of 5-HT we have not observed significant changes in the neurotransmitter accumulation.

Surface modification, as achieved by elevation of GM_1 could influence the hydrophobic region of the membrane by increasing the Ca^{2+} binding capacity. In fact, N-ase treatment was associated with moderate increase in calcium content as visualised by CTC (chlorotetracycline), a lipophilic fluorescent chelate probe. However, the exogenous GM_1 have not affected the calcium content in the synaptoplasmatic membrane.

It has been proposed that gangliosides introduced or metabolically generated in the synaptoplasmatic membrane may specifically interact with certain step of neurotransmission.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (377—380)

CEREBRAL CONSEQUENCES OF EXPOSURE TO HYPEROXYGENATION IN RATS

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Several studies have reported that peroxidation of unsaturated fatty acids, a free-radical chain reaction, may occur in biological systems [1], especially biomembranes [2], causing structural and functional alterations. Various products are formed during lipid peroxidation: hydroperoxides, carbonyl compounds and conjugated double bounds-intermediates in the reactions of free radical oxidation of fatty acids.

As was reported previously [3], the uptake of labelled dopamine is enhanced by prior exposure of the synaptosomes to Fe^{2+}, which gives rise to peroxidation of the synaptosomal lipids in vitro. To examine further the relationship between the dopamine metabolism and peroxidation in the present study, the biochemical changes in CNS were induced in vivo by normobaric hyperoxia.

Male Wistar rats (180–220 g) were used. The animals were exposed to 100% oxygen, the brains were withdrawn at the time intervals indicated and rapidly homogenized in a modified Kreb's-Henseleit saline. The contents of malonaldehyde (MDA), endogenous dopamine and protein sulfhydryl groups, were determined in homogenates from cerebral cortex, whereas the uptake of dopamine was measured in synaptosomal fraction. The conjugated double bounds (CDB) and lipids fluorescence were determined in total lipid extracts of brain cortex.

As shown in Fig. 1 *B*, the rate of dopamine uptake in hyperoxic rats was markedly increased in both the rapid and the slower phase, the maximal stimulation being reached after 5 h hyperoxia. The enhancement of dopamine uptake correlated with the rise in the level of endogenous dopamine in the brain (Fig. 1 *A*), as well as with an increase of CDB and lipid fluorescence (Fig. 1 *D*, *F*). On the other

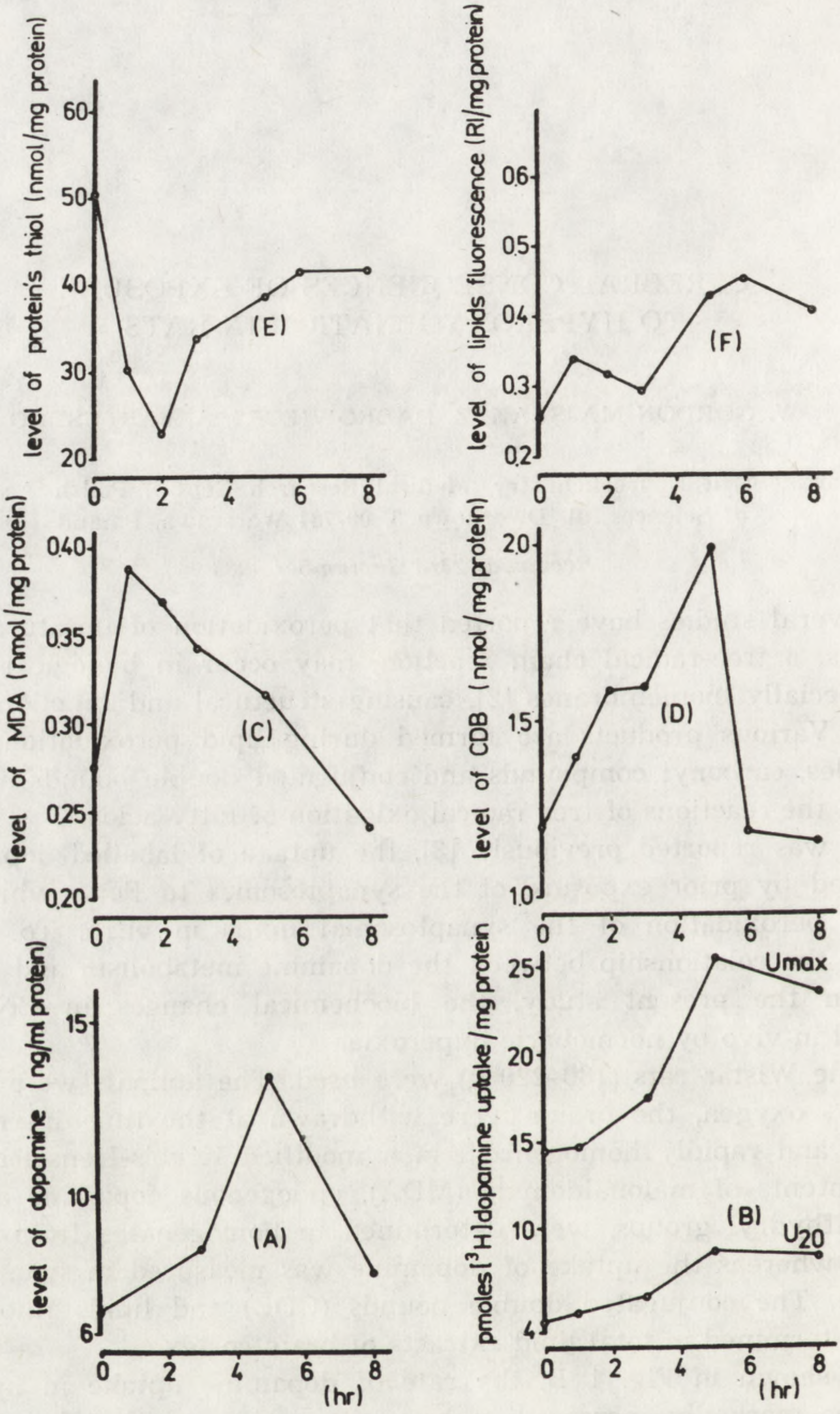

Fig. 1. Cerebral consequences of exposure to hyperoxygenation in rats

hand, the rapid lipid peroxidations monitored by MDA production (Fig. 1 *C*), correlated well with parallel fall in protein thiols group. It should also be mentioned that the level of the SH group as well as MDA production, had returned to the control value during the prolonged hyperoxygenation. It is generally considered that hyperoxia enhances $O_2^{\cdot-}$ production and induces the synthesis of superoxide dismutase. Lipid peroxidation occurs when peroxide, generated by $O_2^{\cdot-}$ and SOD, initiates a chain reaction involving alkyl radicals, and finally results in the destruction of polyunsaturated fatty moieties in the membrane phospholipids. A similar radical mechanism is believed to be responsible for the oxidation of thiol groups.

The increase in the rate of dopamine accumulation probably reflects the conformational changes in the carrier and/or its environment in the membrane. The results of the present study show that metabolism of dopamine and peroxidation of biomembranes follow a parallel change after in vivo exposure to hyperoxygenation.

In conclusion, we suggest that some neurophysiological mechanisms in the brain may be mediated by reactive species of oxygen.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (381—382)

MATHEMATICAL MODEL OF THE CELL-CELL SPECIFIC ADHESION

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A theory of the aggregation of cells in Poiseuille flow is presented. It concerns situation when the cell-cell adhesion is mediated by bonds between specific molecules on the cell surfaces (such as lectin and carbohydrate or antigen and antibody). In the process of cell adhesion by lectins three distinct procesess may be distinguished,

(a) diffusion of molecules and attachment to the cell surface (adsorption),

(b) rearrangement of adsorbed molecules resulting in a equilibrium configuration (reconformation),

(c) collisions of cells promoted by Brownian motion or velocity gradient which may result in the formation of aggregates.

In Poiseuille flow the rate of adsorption of lectins on the cell surfaces is much faster than that of cell aggregation [1]. The process of rearrangement of adsorbed lectins constitutes a complex problem involving such parameters as temperature, lectin concentration etc. At low concentration of lectins, however, the distribution of adsorbed molecules on the surface of normal cells is mostly uniform [2].

As a measure of probability of cell sticking in the flow conditions, the capture efficiency coefficient a_0 is used. It has been shown [1] that the reduction in the initial cell concentration N_0 by aggregation during Poiseuille flow is given by the equation:

$$N/N_0 = \frac{4\int_0^{x_k} x\,(1-x^2)\exp\left(-\frac{kxz}{(1-x^2)}\right)dx}{x_k^2(2-x_k^2)}$$

where

$$x_k = \frac{R\pi}{V_0}\left(\frac{2H(D_a+D_b)n_a n_b d_0 f}{8.16\mu\ bF(d_0)}\right)^{1/2}; \qquad k = \frac{8a_0\varphi_0}{P\pi}$$

and z is the length of the tube, φ_0 is the volume fraction of cells in the suspension, R is the tube radius, H is the constant by which the reaction rate falls short of its diffusion limit [3], n_a, n_b are the numbers of complementary receptors per unit area on colliding cells, D_a, D_b are the diffusion constants for motion in the plane of the membrane, b is the cell radius and d_0 is the maximum distance between the cell surfaces at which aggregation can take place.

In order to test the above approach, measurements of the aggregation of the rat thymocytes with lectin *Ricinus communis* were performed. The results of the experiments are presented on Fig. 1. According to the above equation, the curve was drawn through the experimental points with the use of the method of the least squares. The best fitting was obtained for the maximum distance between cell surfaces $d_0 = 5.5$ nm. This value may be compared with the dimension of the molecule of *Ricinus communis* which is equal to 7.8 nm. This fact suggests that the receptor sites for *Ricinus communis* on the surfaces of rat thymocytes are placed within the cell membrane.

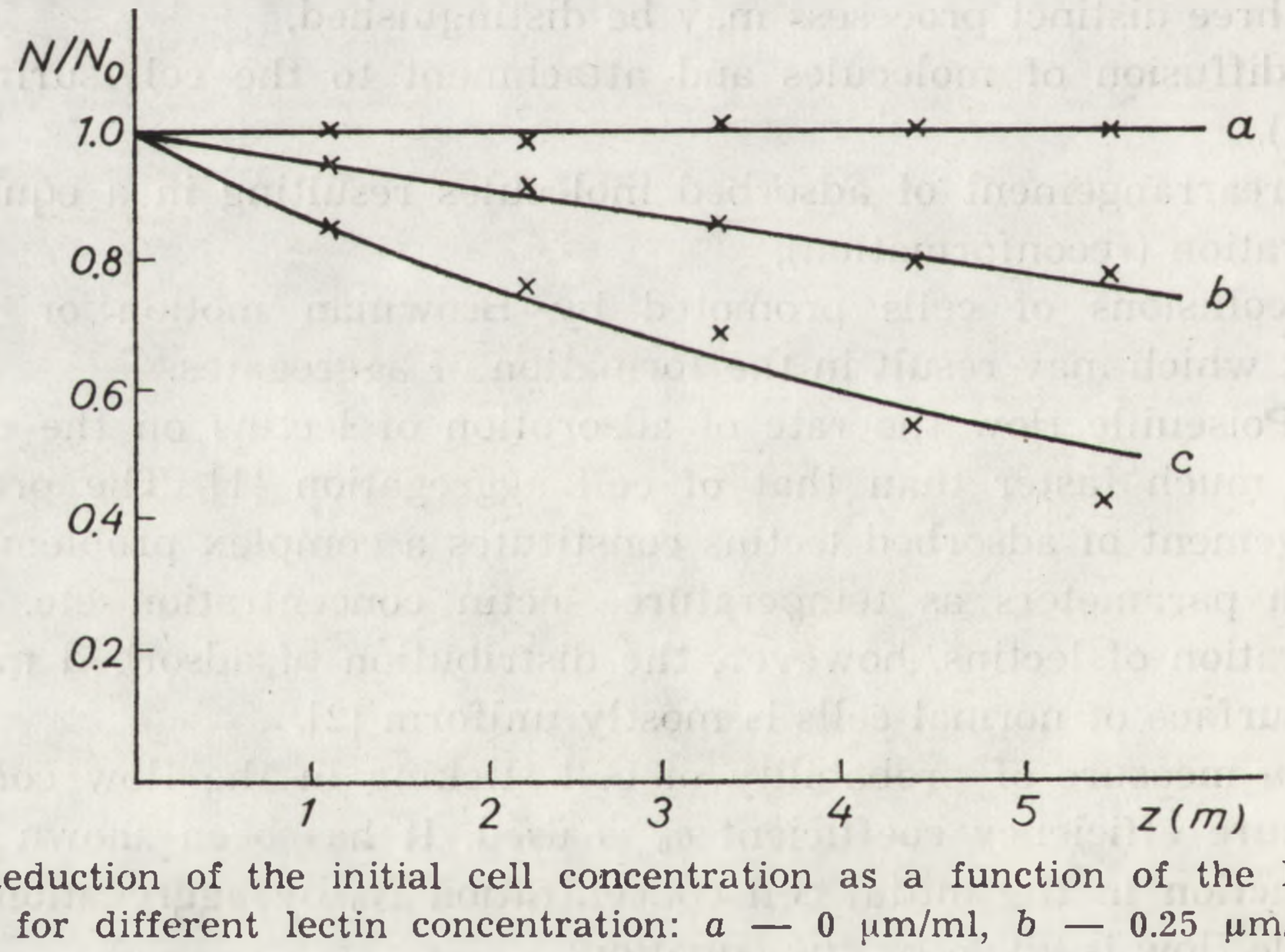

Fig. 1. Reduction of the initial cell concentration as a function of the length of the tube for different lectin concentration: *a* — 0 μm/ml, *b* — 0.25 μm/ml, c — 4.65 μm/ml

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (383—386)

EFFECTS OF FREE FATTY ACIDS ON SYNAPTOSOMAL NEUROTRANSMITTER TRANSPORT SYSTEMS

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Peterson et al. [1] showed that bovine serum albumin (BSA) markedly stimulated the Na^+-dependent uptake of proline by synaptosomal fraction from rat brain, and that several other proteins had no such effect. Stimulation of this uptake by BSA was connected with the same peptide fragment of BSA, which binded long-chain fatty acids to BSA. Authors suggested that the stimulatory effect of BSA was related to its capacity to bind free fatty acids. More recently Rhoads et al. [2] determined, that the stimulatory effect of BSA was not specific for proline, but also occurred with other Na^+-dependent amino acid uptake systems.

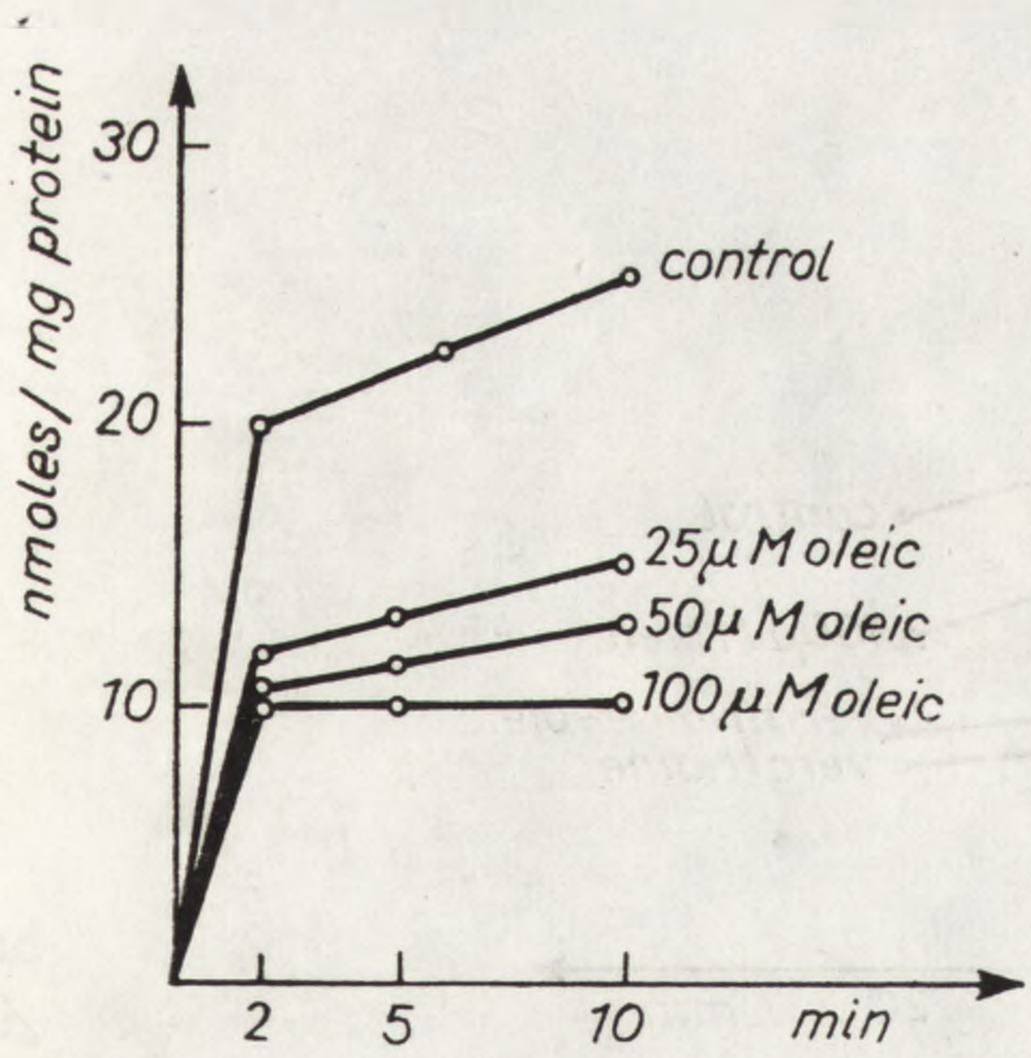

Fig. 1. Effect of oleic acid on uptake of dopamine

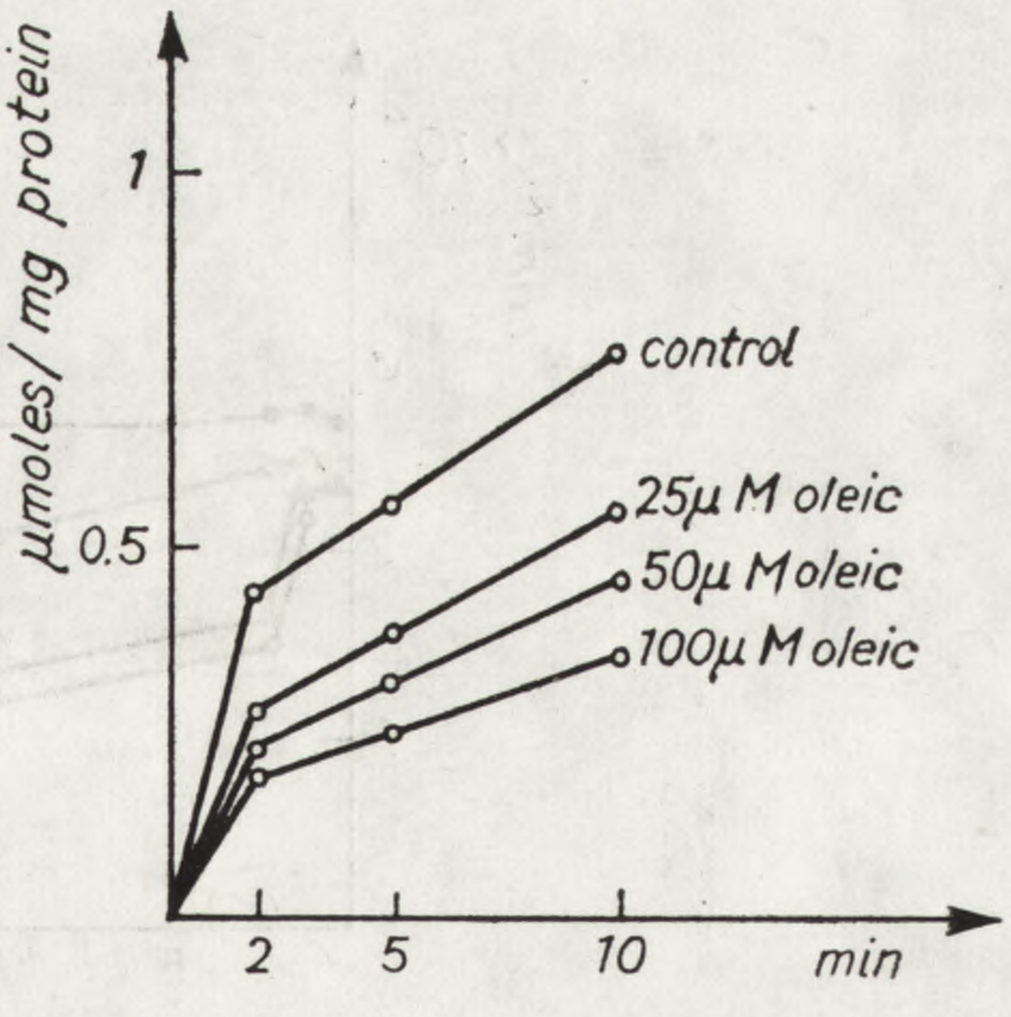

Fig. 2. Effect of oleic acid on uptake of GABA

In the present study we investigated the effects of representative saturated and unsaturated fatty acids on Na^+-dependent neurotransmitters uptake and release systems in synaptosomal fraction of brain. Synaptosomal fraction was isolated from hemispheres of rats brain by flot-

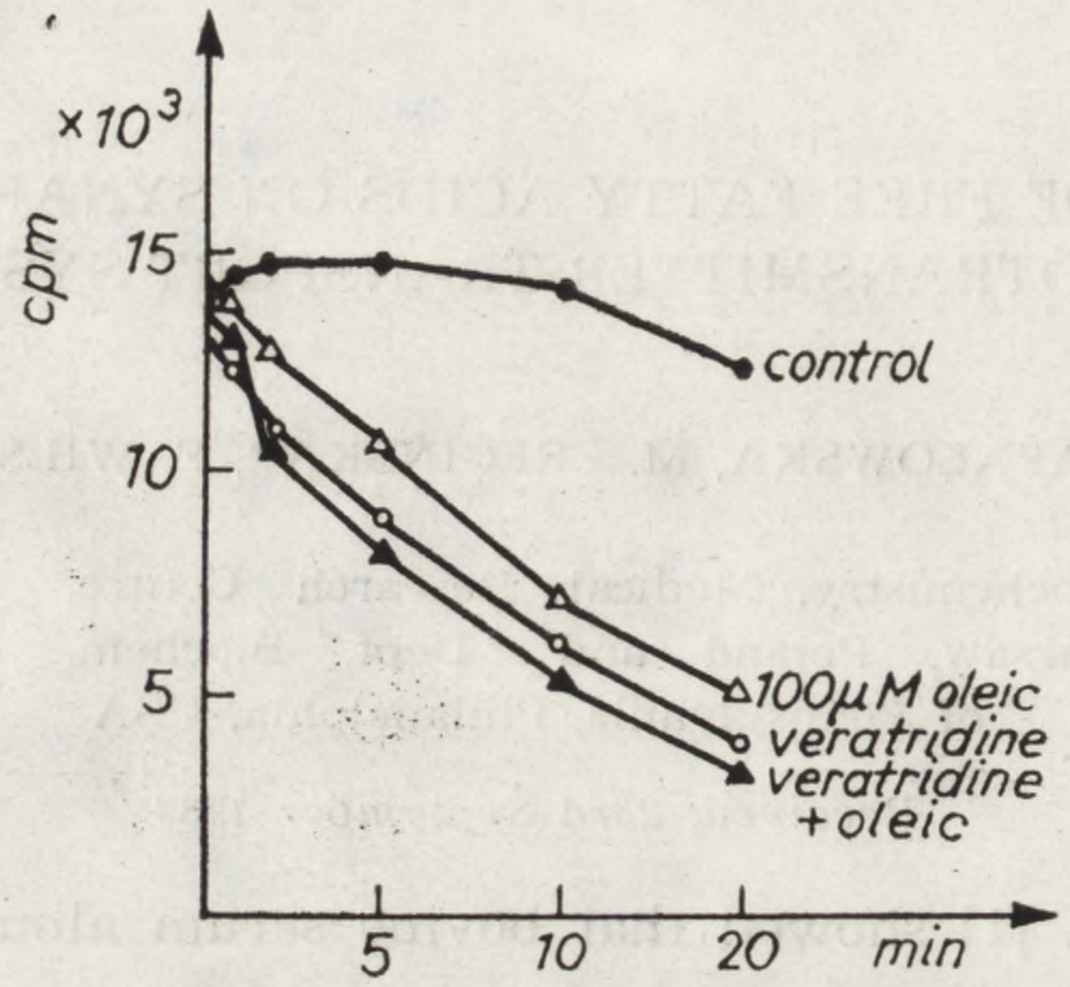

Fig. 3. Effect of oleic acid and veratridine on efflux of dopamine

ation method in the Ficoll gradient [3]. The uptake measurements started by the addition to synaptosomal suspension the appropriate radioactive neurotransmitters: ^3H dopamine and ^{14}C GABA. Samples were withdrawn at the time intervals and centrifuged through a layer of silicone oil. Radioactivity was counted in the supernatant and in the pellets. The measurement of neurotransmitter release was performed after the preincubation of synaptosomes with radioactive material. The up-

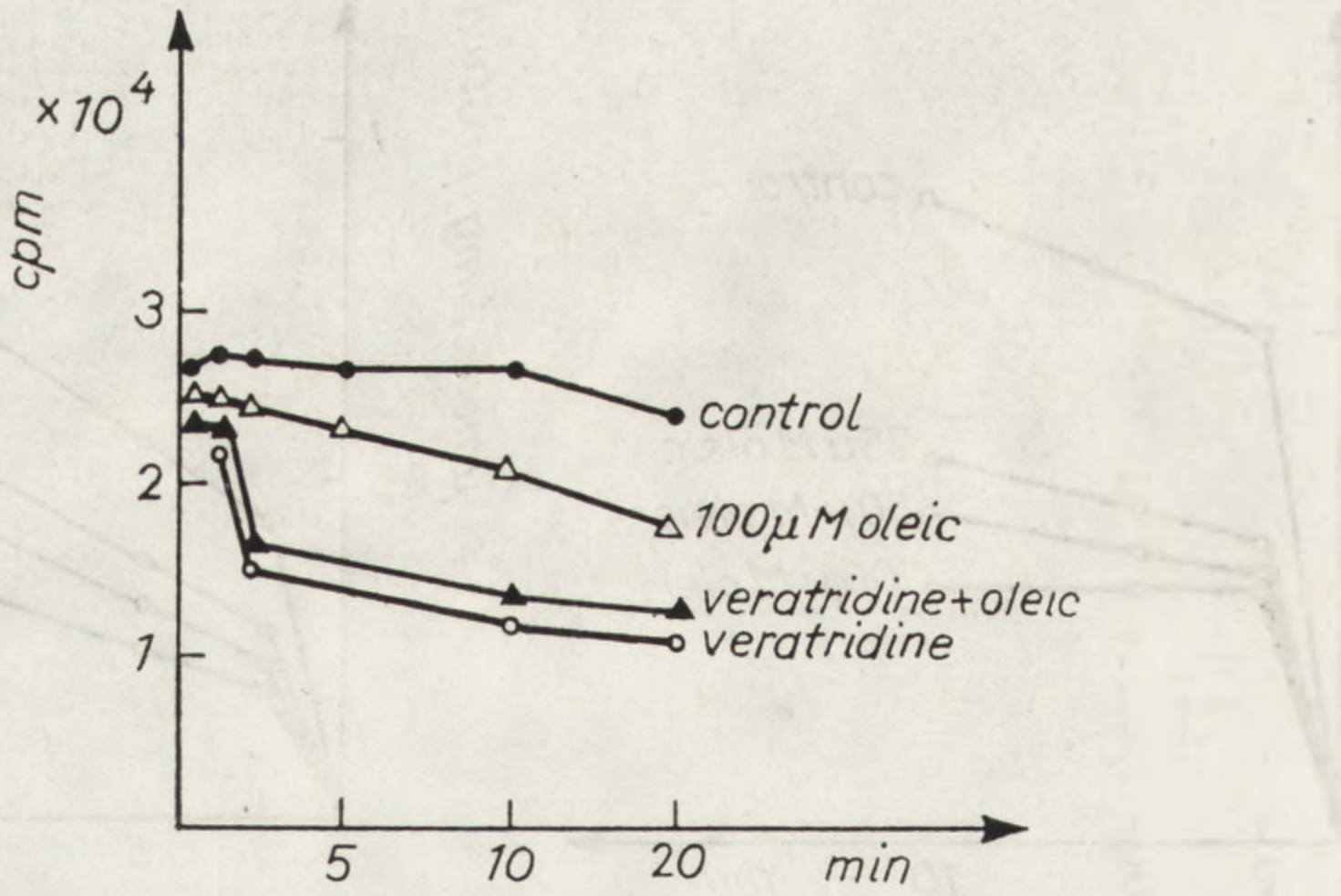

Fig. 4. Effect of oleic acid and veratridine on efflux of GABA

take reaction was stopped by 10-fold dilution of incubation mixture. Samples were removed at the time intervals and centrifuged through the layer of silicone oil, and radioactivity was counted like in the case of uptake measurements. We have found, that oleic acid decreased up-

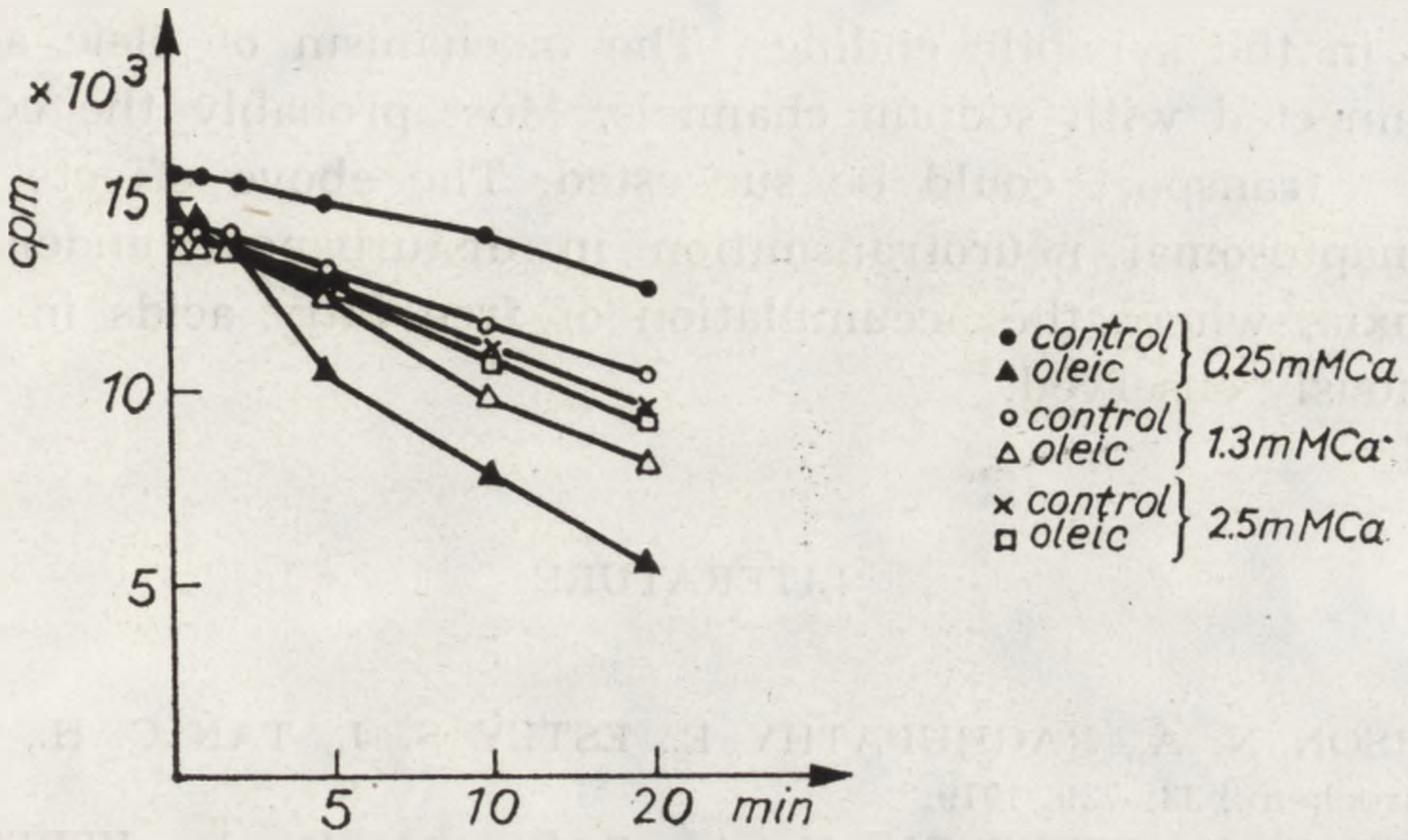

Fig. 5. Calcium dependent increase of dopamine release, caused by oleic acid

take of dopamine about 60% and GABA about 50%. This inhibition was a function of time and oleic acid concentration (Figs. 1 and 2). Palmitic acid gave only small inhibitory effect. Inhibition of neurotransmitters uptake by oleate was independent on Ca^{++} concentration and tetrodotoxin (TTX) didn't reduce this inhibition.

The oleic acid (contrary to palmitic) strongly activated release of dopamine (70–80%) and GABA (20–30%) from synaptosomes (Figs. 3

TABLE 1

Effect of oleate and palmeate on intrasynaptosomal H_2O volume and concentration of K^+

Fatty acids concentration μM	H_2O vol.		$[K^+]$ mM		$[K^+]_0 / [K^+]_i$	
	oleic	palm.	oleic	palm.	oleic	palm.
0	1.05	1.01	50	53	9.6	10.8
25	1.05	1.09	48	58	9.4	11.6
50	1.00	1.04	49	54	9.6	11.0
100	1.09	1.05	55	57	10.1	11.4

and 4). This effect was dependent on Ca^{++} concentration (Fig. 5). TTX, which reversed the veratridine inhibition, had not any effect on inhibition, produced by oleic acid (Figs. 3 and 4). The inhibition of the

uptake and stimulation of the efflux of neurotransmitters from synaptosomes caused by oleic acid, is not connected with detergentlike effect of free fatty acids (concentration of K^+ ions and volume intrasynaptosomal water didn't changed in our experimental condition (Table 1). Our data showed that oleic acid strongly influenced the neurotransmitter transport in the synaptic endings. The mechanism of oleic acid effect is not connected with sodium channels. Most probably the conjunction with Ca^{++} transport could be suggested. The above effects could involve synaptosomal neurotransmition in disturbances under ischemia and hypoxia, where the accumulation of free fatty acids in the brain was previously observed.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (387—390)

CHANGES OF TRANSPORT ACTIVITIES OF THE INTESTINAL EPITHELIUM UNDER THE INFLUENCE OF BIPYRIDYL DERIVATIVES

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Diquat and paraquat are characterized by similar chemical structure as well as common mechanisms of action directed against plants. It is supposed that it consists in the destruction of membranes of plant cells by free bipyridylium radicals. Both have proved to be toxic for mammals. The action of paraquat concerns chiefly the pulmonary tissue. Diquat do not cause lesions of pulmonary epithelium described in the case of the preceding preparation. But negative influence on the intestinal tract was observed. The presented investigation were carried out for the determination of the action of diquat and paraquat on transport activities of the intestinal epithelium in conditions in vitro through the analysis of bioelectrical parameters as well as morphological investigation.

As material for investigation the final part of the small intestine of the white New Zealand rabbits was used. Preliminary preparation of tissue, and also the method of the calculation of bioelectrical parameters (potential difference-PD as well as resistance-R) have been described earlier [1]. Paraquat or diquat have been introduced into medium (Hanks' fluid) on mucosal side, after stabilization of PD and R of the intestine (about 1 hour). Two concentration of these compounds were used: 10^{-4} and 10^{-3} mol/l. After the administration of one of the mentioned preparations, bioelectrical investigations were continued during the period of 90 min. Analogous experiments were carried collaterally, during which tissue was collected for morphological investigations. The fixed preparations were analysed in the electron microscope.

The differentiated influence of diquat on the epithelium of the

rabbit's small intestine must be underlined. This preparation with the concentration 10^{-4} mol/l caused temporary decrease of the value PD of the investigated tissue. In the case of higher concentration, it caused reciprocal effect (compare Fig. 1 and Table 1). This differentiation has

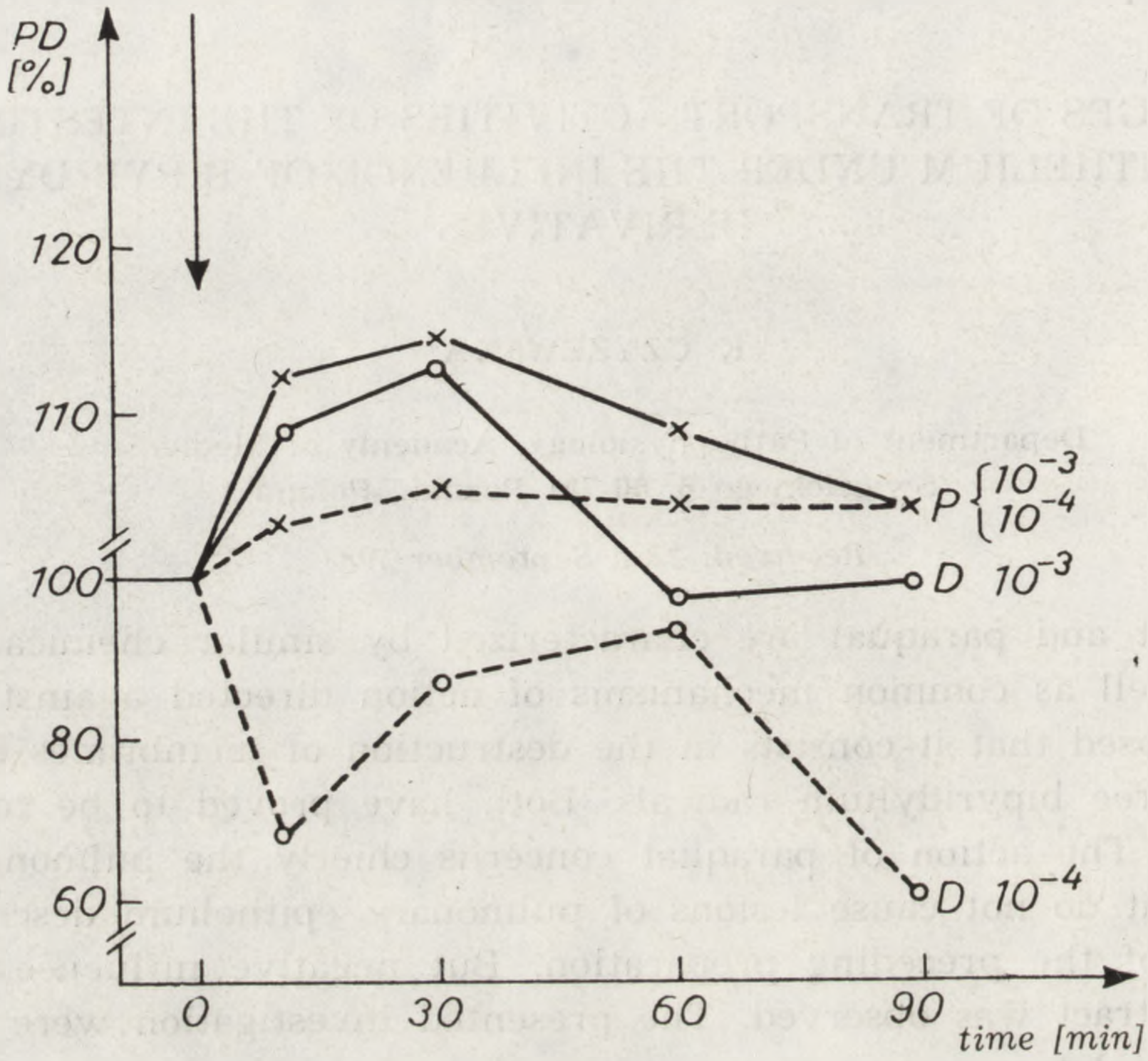

Fig. 1. Changes of the value of the potential difference (PD) of the rabbit's small intestine under the influence of bipyridyl compounds (in % of control values). ↓ moment of the administration of pesticide continuous line means concentrations 10^{-3} mol/l, dashed line means concentrations 10^{-4} mol/l of paraquat (x) and diquat (o)

confirmed the results of morphological investigations. In the case of the action of diquat in concentration 10^{-3} mol/l, the dilatation of intercellular spaces of the intestinal epithelium was observed, when lower concentration did not cause changes. Whereas paraquat caused temporary increase of transport capatities of the investigated tissue (increase of the value PD as well as slight decrease of R) in the case of both used concentration (compare Fig. 1 and Table 1). But disorders in the morphologic picture of the intestinal epithelium were not observed.

Conclusions: 1. Changes of transport activities (value PD) of the intestinal epithelium under the influence of diquat and paraquat confirm the membranous direction of these compounds. 2. The differ-

TABLE 1

Influence of bipyridyl compounds on bioelectrical values of the small intestine of rabbit (in % of control values)

Name of compound	Concentration in mol/l	Analysed values	Time of measurement after administration of pesticide (in minutes)			
			10	30	60	90
Diquat	10^{-4}	PD	68.2±7.3	87.1±7.8	93.0±5.4	59.7±12.3
		R	105.5±4.2	105.5±6.7	91.5±3.1	87.6±5.3
	10^{-3}	PD	109.1±3.3	112.7±13.1	97.9±17.3	99.2±15.8
		R	101.6±4.3	99.8±2.1	98.1±2.6	98.2±3.3
Paraquat	10^{-4}	PD	102.7±4.0	105.2±8.2	104.0±12.6	103.6±18.1
		R	98.8±0.9	98.2±1.6	97.6±1.6	95.1±3.0
	10^{-3}	PD	112.4±2.6	114.6±4.3	109.1±6.7	102.9±8.7
		R	97.8±1.1	95.4±0.8	95.4±1.3	93.8±1.8

As control value was accepted the average from 3 measurements at 30, 20 and 10 minutes before the administration of pesticide.

Note: mean value ± standard error are presented.

entiated, according to the used concentration, influence of diquat on transport functions of the small intestine can indicate the complex way of its action within the plasmatic membrane. 3. The described informations emphasize the separate action of diquat and paraquat. This concern, in this case, the intestinal epithelium.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (391—394)

FEATURES OF THE PARIETAL PERITONEUM AS A "LEAKY" MEMBRANE

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In basic studies on improving technique of treating patients with renal failure with peritoneal dialysis, one of the most important elements is evaluation of physiological characteristics of the peritoneum. Since the peritoneal mesothelium has monolayer structure with typical intercellular junctions, one cannot exclude that there exist in it transport processes characteristic of the epithelium. These studies were done to measure the basic electrophysiological parameters and the ion permeability in order to compare them with other, well investigated models of "leaky" epithelia.

The studies were performed on isolated pieces of rat and rabbit diaphragm after desquamation of the pleural mesothelium layer by the technique described previously [1]. The values of the potential difference (PD) and the resistance (R) for the peritoneal mesothelial layer (pt) were stated from the difference obtained after removing it by method of incubation with sodium deoxycholate (dxch) in concentration: of 5 mM and 2.5 mM for rat and rabbit tissue respectively. During a typical experiment with diaphragmatic parietal peritoneum of rat and rabbit, TRIS replaced Na ions, either on the peritoneal side (meso), or on the removed pleural side (vasc). The ion transfer (Φ) was determined by radioisotopical technique using ^{86}Rb as analog of K ions. 2,4-dinitrophenol (DNP) in concentration of 1 mM was added on vasc side, and effect of PD and R change were measured.

Removal of the pleural mesothelial layer from the piece of an isolated rat diaphragm, resulted each time in a potential difference with a positive change on the peritoneal mesothelium side. Fig. 1 presents the typical course of potential change from a chosen experiment. The same

change range of PD was noted when the peritoneal mesothelium was removed in the 3rd h of the experiment. This effect on rabbit peritoneum was indeterminable (Table 1), but changes of other parameters, as R and increase of ion permeability (mean ± SE), which resulted

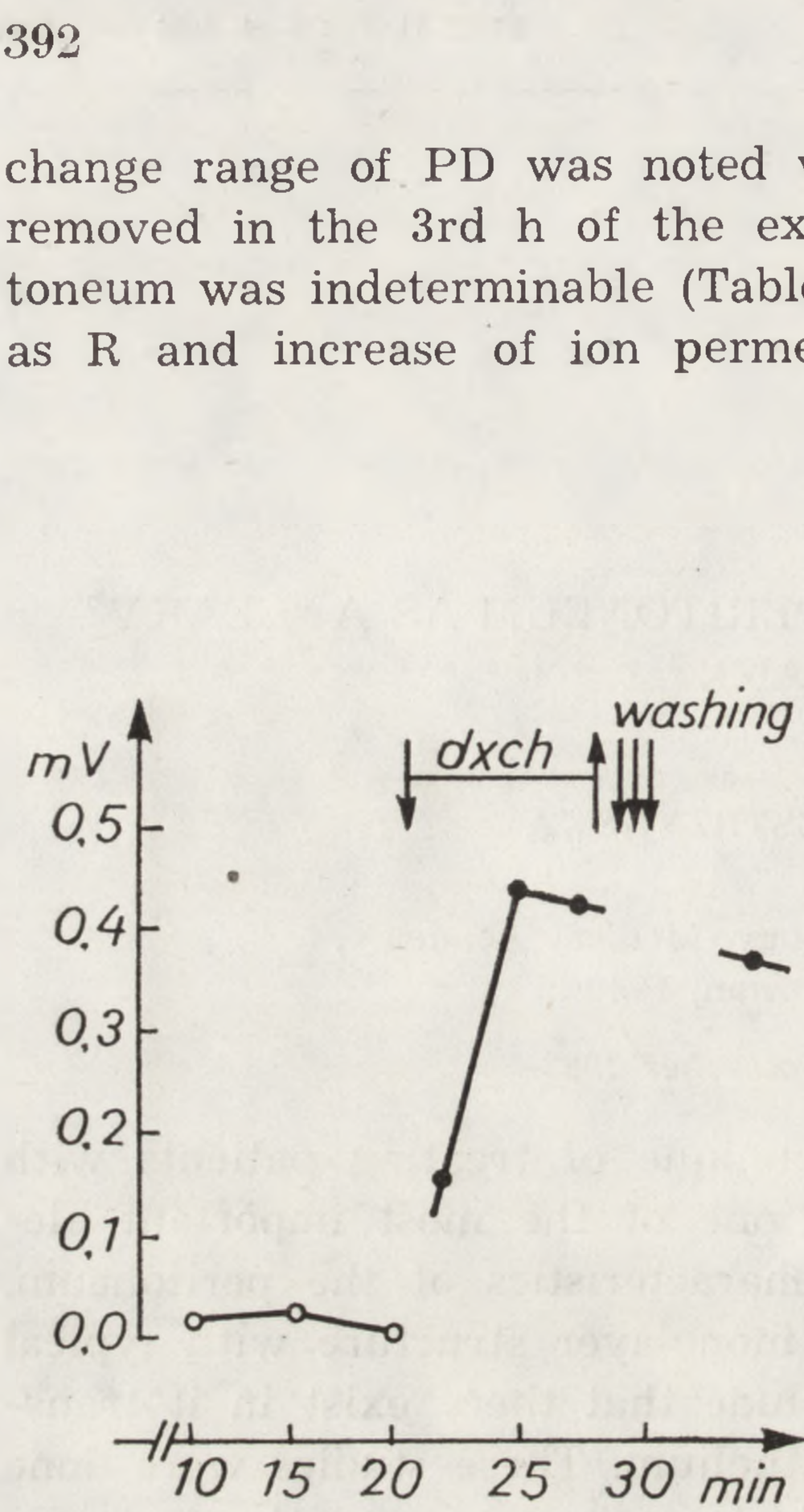

Fig. 1. PD changes during removal of pleural mesothelium of rat diaphragm on a chosen experiment. Values of PD result from parietal peritoneal mesothelium function; o — value of PD in the 20th min. of experiment taken as base $PD_{20} = 0{,}0$), ● — values of PD as differences from $PD_x - PD_{20}$

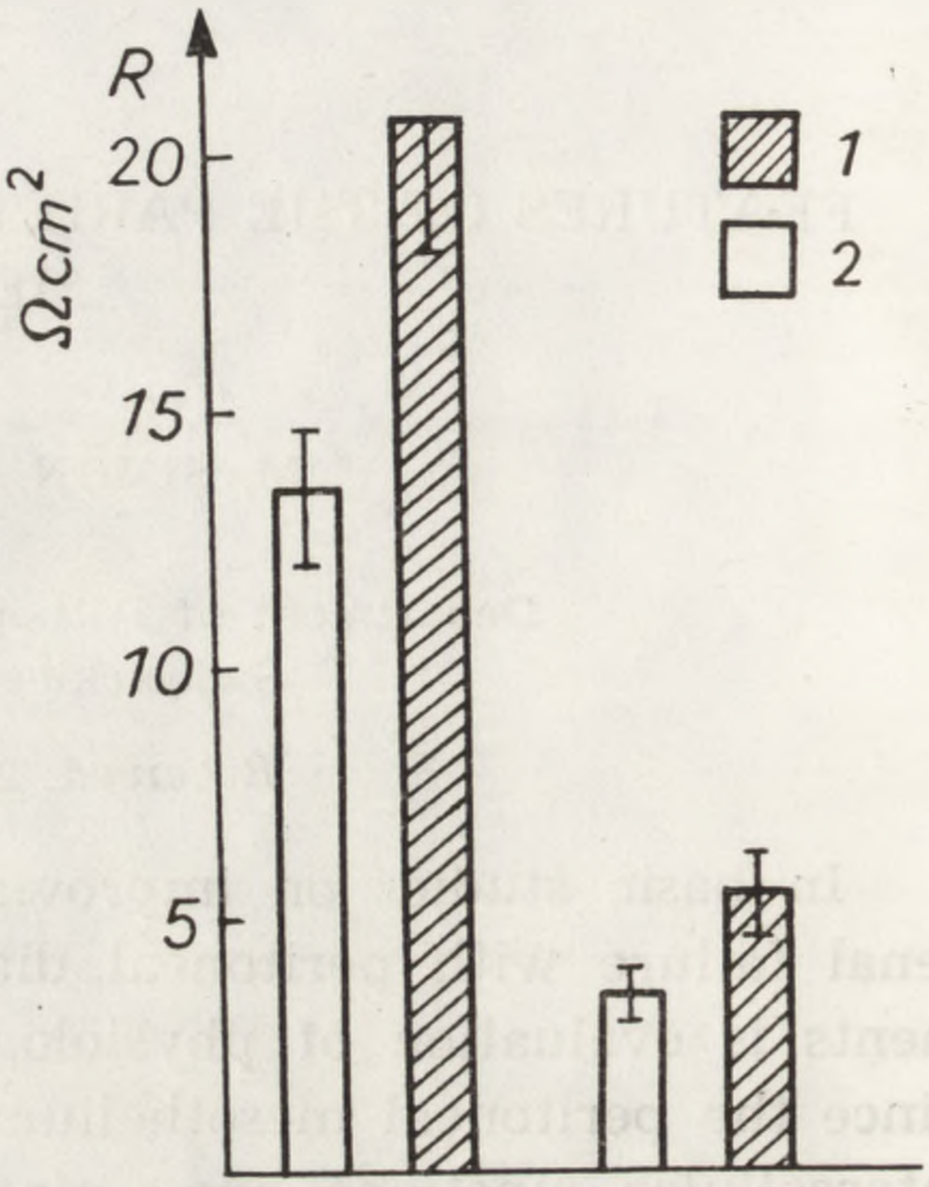

Fig. 2. Resistance increase of peritoneal mesothelium of rat and rabbit after adding DNP; *1* — after DNP, *2* — control

from the peritoneal mesothelium removal, were comparable in tissues of both animals. One of the signals of the epithelial ionoselectivity are measurements of ΔPD, when sodium ions in the medium are replaced by other molecules e.g. TRIS. Table 2 presents mean values of ΔPD for both tissues in both variants of ion arrangement. In another series of experiments, inhibition effect of added DNP was determined. Increase of resistance values for the parietal peritoneal mesothelial layers of rat and rabbit is shown in Fig. 2. In this study changes of PD are not significant statistically.

C o n c l u s i o n s: The results described here show the role of the parietal peritoneal mesothelium as a barrier, and characterize it as

TABLE 1

Values of the determined parameters: PD and R for the parietal peritoneum of rat and rabbit diaphragm and increase of ion transfer after removing this mesothelial layer

Parameters	pt	
	rat	rabbit
PD (mV)	0.26±0.03 $n = 12$	n.s.
R ($\Omega \cdot cm^2$)	13.6±1.3 $n = 11$	2.9±0.5 $n = 12$
$\Delta\Phi$ ($nmol \cdot s^{-1} \cdot cm^{-2}$)	0.36±0.05 $n = 12$	0.26±0.04 $n = 12$

TABLE 2

Effect of replacement of Na ions by Tris in medium on both sides of the tissue, determined as ΔPD in the 15th min. after medium exchange

Tissue	meso	vasc
rat pt	+0.77±0.21 $n = 9$	−1.18±0.22 $n = 12$
rabbit pt	+0.28±0.16 $n = 5$	−0.54±0.12 $n = 9$

a "leaky" membrane. Though the observed changes of electrophysiological parameters are different in values, they are consistent with direction of changes determined on other epithelia of this group [2]. This should encourage further studies on the nature of transport processes in peritoneum, the knowledge of which can be used to explain pharmacological influence of drugs on peritoneal dialysis, in order to increase its efficiency.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (395—396)

TRANSEPITHELIAL TRANSPORT IN ESTABLISHED CELL LINE (MDCK) CULTURE

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Cultured epithelial cell monolayer for studies relevant to the biological transport was initiated by Misfeldt [1] on the MDCK established line. The MDCK (ATCC CCL 34) cell line was derived from a kidney of an adult female Cocker Spaniel, 1958, by Madin and Darby [2]. In vitro, MDCK cells form monolayer with possesses a typical epithelial structure, and demonstrates net transmonolayer ion transport [3]. Cultures of the MDCK cell line on millipore filter form monolayer with functional properties analogous to those of transporting epithelia. They contact each other to form occluding junctions (tight junctions), which confer to the cell layer the role of an effective permeability barrier, and are polarized i.e. channels, carriers, pumps and hormonal receptors present on the basolateral membrane region are different from those on the apical side. The cell layer generates the spontaneous electrical potential difference. MDCK cells were maintained by serial passages in the glass tissue culture flask with Dulbecco's Modified Eagles Medium + 10% fetal bovine serum. Subcultures were prepared using 0.02% EDTA and 0.05% trypsin. The cultured cells were plated $5 \times 10^5/cm^2$ directly into the Petri dish over the filter. Close monolayer of the MDCK cells appeared 5–7 days later. The millipore filter with the cell monolayer was removed from the culture dish and mounted as a flat sheet between two half chambers. Transepithelial potential difference and short-circuit current (SCC) was measured according to Ussing's method [4]. The value of SCC is dependent on the amount of net transported sodium. Electrophysiological parameter — SCC — achieved the steady-state value after 30 minutes from the moment when the membrane was mounted into the chamber (Fig. 1). Amiloride added to the apical side of culture de-

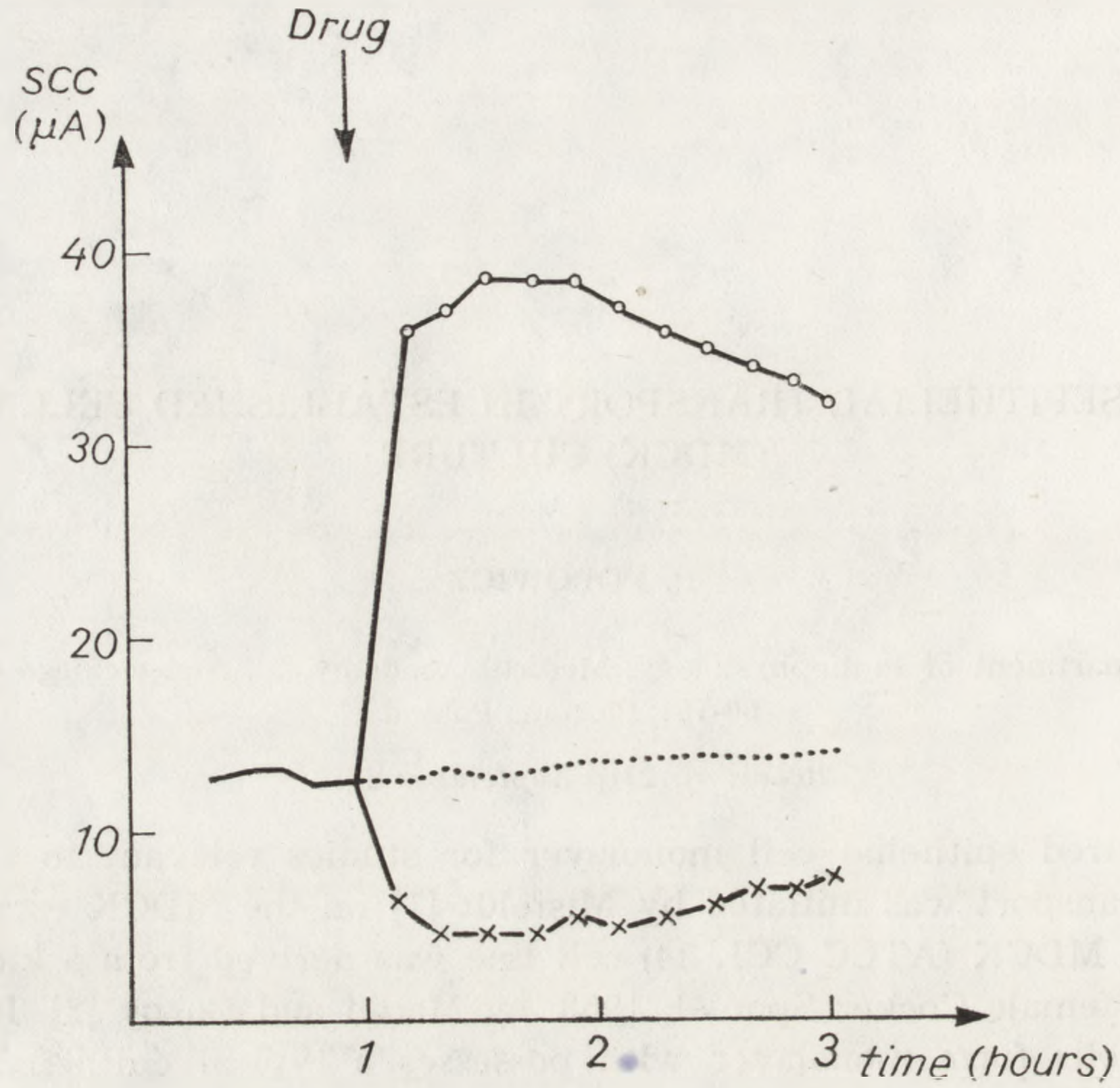

Fig. 1. Changes in the value of short circuit current — SCC — of the MDCK monolayer after: o—o Acetazolamide (10^{-4}M) addition to the medium into the basolateral side of culture ($n = 6$), x—x Amiloride (10^{-5}M) addition to the medium into the apical side of culture ($n = 6$), ... control — no drug was added during the experiment ($n = 6$)

creased SCC to 30%, Acetazolamide added to the basolateral side of culture increased SCC to 320%. Very rapidly developing changes after Amiloride and Acetazolamide administration confirm that MDCK monolayer on millipore filter is an effective barrier.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (397—400)

LIPID METABOLISM IN HYPOGLYCEMIC AND ISCHEMIC BRAIN

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Hypoglycemia was included by Brierley [1] in the hypoxic pathologies, but it seems that it ought to be separate. Previously it was postulated that pathogenesis of hypoglycemia is similar to ischemic and hypoxic insults. Actually, Kalimo et al. [2] and Olsson et al. [3] suggested that changes associated with hypoglycemia may have distinct pathological manifestation. Although both ischemic and hypoglycemic conditions elicited an increase in brain FFA level, other differences in lipid metabolic changes can be observed. Until now little information has been available on the status of lipid metabolism in the brain during hypoglycemia. Special attention should be placed on investigations of the metabolism of phospholipids, since they are important in regulating neuronal function. In the present study the effect of hypoglycemia on phosphatidylinositol metabolism in brain cortex synaptosomes was investigated.

Hypoglycemia was produced by intraperitoneal injection of 1 μ of crystalline insulin per 100 g b.w. The animals were used for the experiments 3 hours after insulin injection, when the level of blood glucose was decreased to 10–20 mg%. Synaptosomes were isolated by the method of Booth and Clark [4]. Inositol incorporation into phosphatidylinositol was measured with myo-[2-^{3}H]-inositol 0.3 μCi and "cold" myo-inositol at final concentration of 5 mM. The incubation mixture contains also 10 mM $MgCl_2$, 1.6 mM CTP and 1.0 mg protein in total volume of 1.0 ml of Krebs-Ringer solution. Lipids were extracted according to Folch et al. [5] and separated by two-dimensional TLC procedure. Radioactivity of the lipid samples was measured in Beckman

LS 900 scintilation counter. Incorporation of myo-[2-^{3}H]-inositol into phosphatidylinositol can occur by synthesis the novo and by inositol exchange and [6, 7]. The present results indicate that synaptosomes actively incorporated myo-[2-^{3}H]-inositol into phosphatidylinositol in the presence of Mg^{2+} and CTP. The CTP dependent inositol incorporation is seven times more active than inositol exchange reaction. Activity of this reaction depends on concentration of protein and time of reaction. CTP-dependent inositol incorporation is inhibited by calcium ions, but CTP independent route is not affected. The incorporation of myo-[2-^{3}H]-

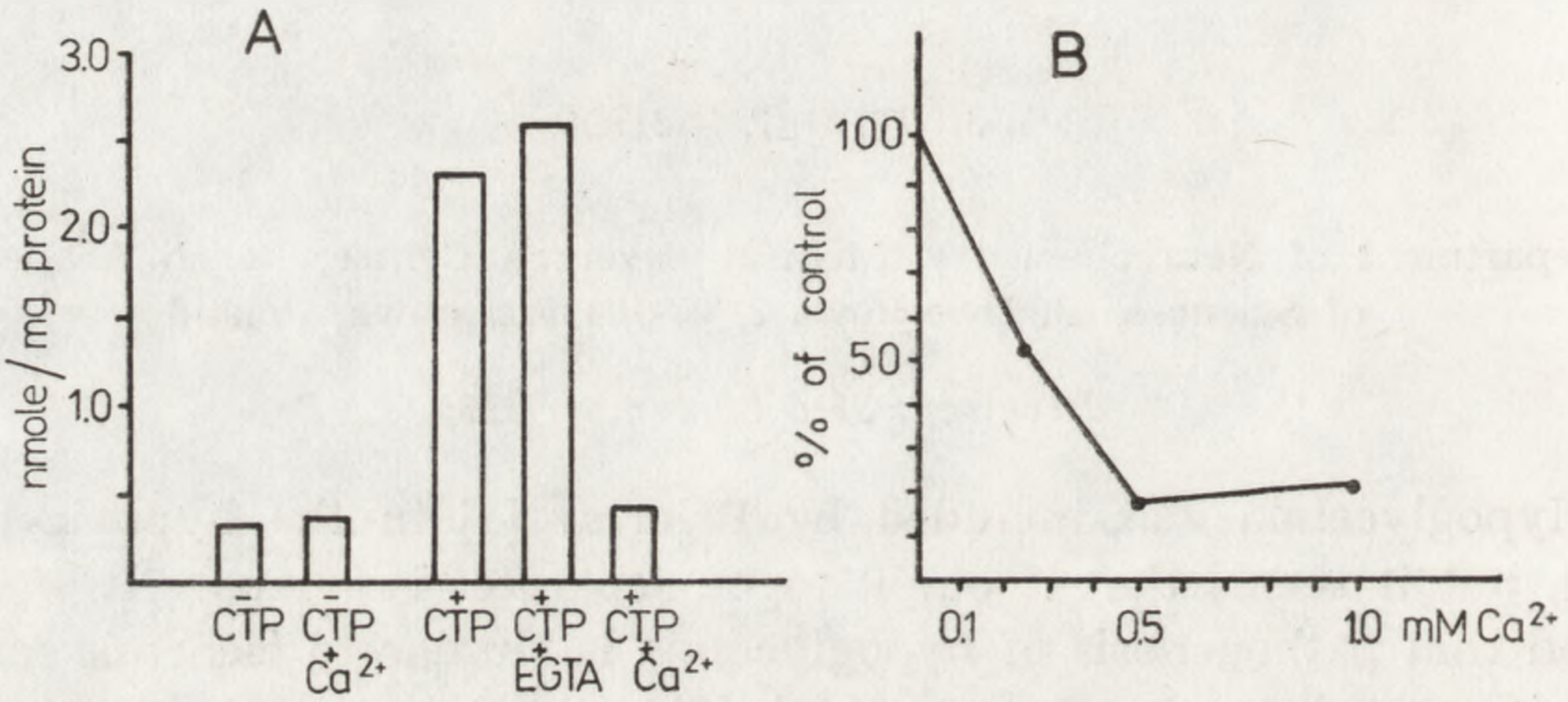

Fig. 1. Effect of Ca^{2+} on myo-[2-^{3}H]-inositol incorporation into PI

-inositol in the presence of CTP and $MgCl_2$ is enhanced by about 30% if endogenous Ca^{2+} is chelated by EGTA (Fig. 1 *A*). In the presence of 0.5 mM $CaCl_2$, 1.6 mM CTP and 10 mM $MgCl_2$ inositol incorporation into phosphatidylinositol occurs at around one fifth of rate observed in the absence of added Ca^{2+} ions (Fig. 1 *B*). The CTP-dependent inositol

TABLE 1

Effect of hypoglycemia on myo-[2-^{3}H] inositol incorporation into synaptosomal phosphatidylinositol

Specification	Control	Hypoglycemia
	nmole/mg protein	
− CTP	0.35±0.04 [3]	0.45±0.04 [5]
+ CTP	2.30±2.20 [3]	1.99±0.34 [6]

The values are the mean ± SD from 3—6 animals.

incorporation in brain cortex synaptosomes isolated from the animals submitted to hypoglycemia, is slightly inhibited by about 14%, but CTP--independent inositol incorporation is stimulated by about 30% (Table 1

and Fig. 2 *A*). This stimulatory effect of hypoglycemia was exclusively observed in mono-phosphatidylinositol, but not in di- and tri-phosphatidylinositol (Fig. 2 *B*). The stimulatory effect on CTP-independent inositol incorporation was not observed in ischemic synaptosomes. Unlike the ischemic conditions which is accompanied by an increase in both FFA and diglycerides (DG), the DG level in brain cortex synaptosomes after induced hypoglycemia, was not altered. Diglyceride might come from polar lipids through phospholipase C activity on diacyl-GPI [3]. This enzyme seems not to be activated in hypoglycemia. These results indicate that ischemia and hypoglycemia differently affected phosphatidylinositol metabolism in brain synaptosomes.

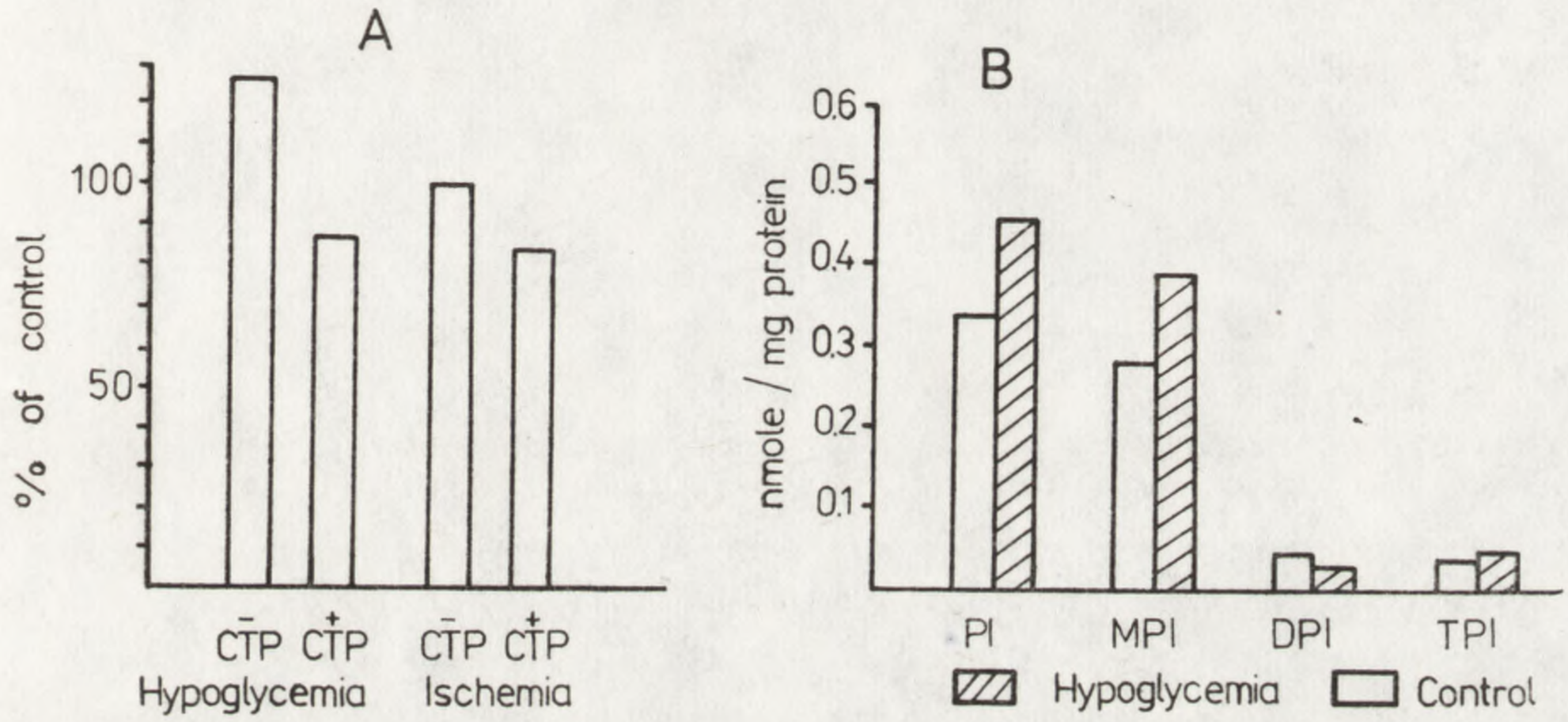

Fig. 2. Effect of hypoglycemia and ischemia on myo-[2-^{3}H]-inositol incorporation into synaptosomal phosphatidylinositol

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (401—404)

STIMULATION OF SECRETION AND PHAGOCYTOSIS IN *TETRAHYMENA*. STUDY ON THE ROLE OF THE CELL SURFACE COAT

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Free living ciliate, *Tetrahymena thermophila*, after conditioning becomes competent to perform a sequence of physiological activities: a massive discharge of secretory granules leading to the formation of pseudocyst, followed by an excystation and the start of phagocytosis. This physiological sequence may be induced by the chemical stimulation with an Alcian Blue (AB) dye [5]. The aim of the present report was to characterize the physiological effects of another polycationic dye, Ruthenium Red (RR) of some sibling characters to AB [4].

Tetrahymena thermophila, stock SB-210 grown in 2% proteose peptone medium supplied, at 30°C [7], at early stationary phase, was conditioned by the washes and starvation for 4–6 hours in an inorganic medium (IM) [1]. Alcian Blue (AB) test (Satir pers. com. slightly modified): 80 µl of cell suspension (density about 10^5 cells/ml) was supplied with 10 µl of 0.2% aqueous solution of AB (8GX Polysciences) for exactly 15 sec, and then diluted with a 0.4% proteose pepton supplied with $FeCl_3$ nad $CaCl_2$ (1 mM) for next 45 sec. After exactly 1 min, cells were either fixed with a drop of formaline and scored for the percentage of cells secreting blue-stained pseudocysts, or they were washed with IM and again periodically assayed with AB test to examine the recovery of ability to secrete the subsequent generation of pseudocysts, the kinetics of excystation and the rate of phagocytosis. As control of phagocytic competence served conditioned cells mechanically stimulated with carmine particles. Ruthenium Red (RR) test were performed exactly as those with AB with 10^{-4} M aqueous solution of a crude RR (Polysciences). Combined AB and RR tests were performed on in vivo preincubated cells in RR for 10 min, then tested with AB. Transmission electron microscope control of secretion was performed on in vivo treated cells with either AB or RR and than fixed with 1% OsO_4 and then routinely proceeded [6].

1. RR in sublethal concentration did not induce secretion of the secretory granules (mucocysts), while AB did (Fig. 1). RR did not inhibit

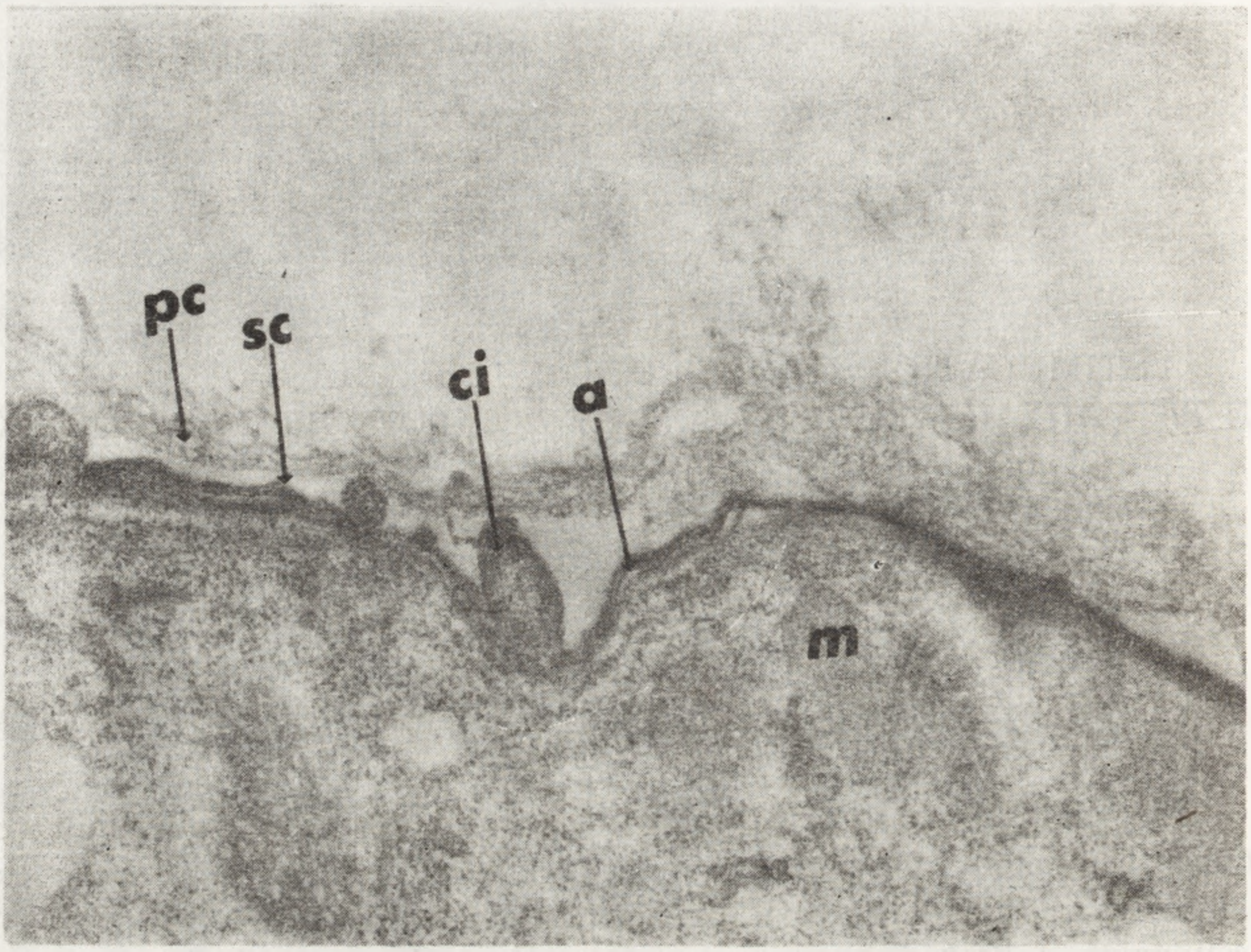

Fig. 1. The cortical area of *Tetrahymena thermophila* after AB stimulation. The layers of pseudocyst (pc) surround whole cell including cilia (ci). The surface coat (sc) is also covered with AB stain. Flattened alveoles (a) and mitochondria (m) are seen below the cell membrane. Magn. 18 000 ×

the normal low-level discharge of some mucocysts (Fig. 2). 2. AB in vivo slightly stained the surface coat of secreting *Tetrahymena*, although such precipitation was detected in EM sections (6 and this report). RR heavily stained in vivo the surface coat, and this complex was detected in EM sections (Fig. 2). RR staining may be washed out with IM. 3. RR staining of the surface coat during preincubation did not affect AB stimulated secretion. Preincubation with RR did not modify the course of the AB stimulated sequence of secretion-excystation and phagocytosis. 4. Preincubation of cells with RR only slightly affected the process of carmine stimulated phagocytosis. 5. AB manifested high affinity to the material extruded from the mucocysts, while the RR did not. AB is highly concentrated within formed food vacuoles. Presence of RR in the medium did not induce its acumulation within the food vacuoles of *Tetrahymena.*

In several ways RR is similar to the AB dye: both are metal containing polycations of high affinity to the similar polyanions of the cell surface coat by electrostatic or ionic forces [4]. There are reports [2,3] that RR also affects the Ca^{++} transport through cell membrane, while

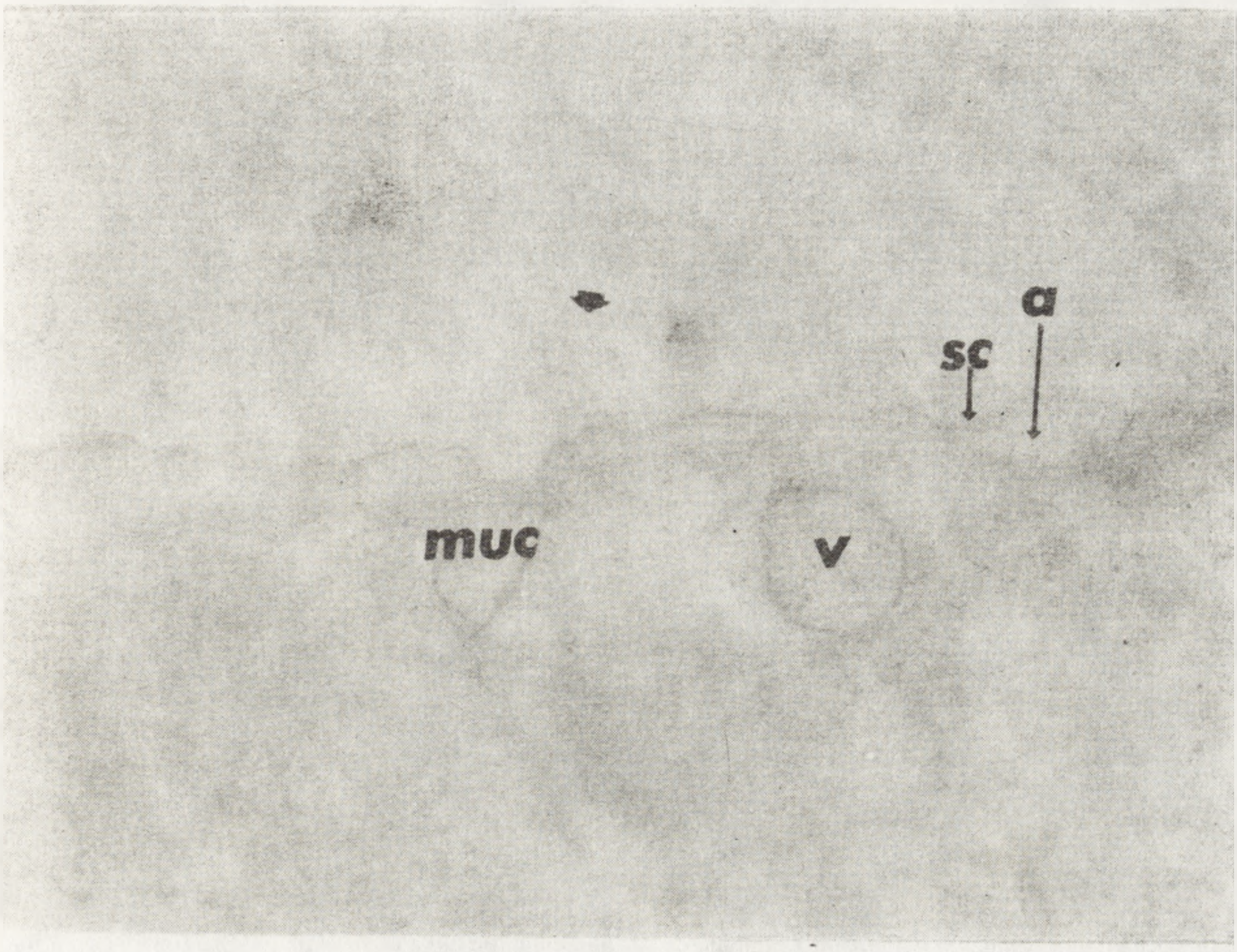

Fig. 2. Unstained section of the cortex of *Tetrahymena thermophila* after in vivo incubation with RR. RR staining is found in a layer of the surface coat (sc). It continues along by the invagination, marking the grazing section through an upper part of secreting mucocyst (muc). Only one vacuole (v) of an uncertain origin is also stained. Other cortical structures, and burst of the secreted mucus (an arrowhead) remain unstained. Magn. 48 000 ×

there is no comparable data on the AB effects. From our results we conclude that precipitation of exposed carboxylated and sulphated residues of *Tetrahymena* surface coat with RR, did not interfere with the specific AB induced secretion, and did not suppress its effector system. Apparently RR complexed with the exposed surface coat reactive groups, did not affect Ca^{++} actions involved in secretion of mucocysts [8].

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (405—408)

STIMULATION AND INHIBITION OF MUCOCYST SECRETION IN *TETRAHYMENA*

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Mucocysts are secretory granules of a ciliate *Tetrahymena thermophila*. They are oval in shape, and surrounded by a limiting membrane. The anterior end of an undischarged, matured mucocyst rests on the outer membrane among the alveolar cisternae of cortex [7]. When a mucocyst discharges, its membrane fuses with the pellicular membrane leaving an opening through which its content is ejected. This degranulation may be elicited by an external addition of a polycationic dye, the Alcian Blue (AB) [8]. This process may be supressed by the presence of Mg^{++} in the medium [9]. AB dye binds to the acidic polysacharides through a salt linkage [6], and this affinity was detected within the surface coat of *Tetrahymena* in post-mortem staining [4]. The question is whether in vivo AB-stimulation, and Mg^{++} inhibition of secretion, may be affected by the experimental modification of the surface coat of *Tetrahymena*.

Tetrahymena thermophila stock SB-210 grown after Orias and Bruns [5] method, at early stationary phase, conditioned by the starvation in inorganic medium [1]. The Alcian Blue (AB) test was performed after Satir (pers. comm.) method, slighty modified [2]. This AB test alone served as control for all other experiments. In experiments, conditioned cells were preincubated during 10 min in the sublethal concentrations of drugs and then their ability to secrete was estimated in the AB test. The following treatments were applied: (1) 15 mM $MgCl_2$ bath, at room temperature; (2) enzymatic digestion with the neuraminidase from *Vibrio cholerae* (Sigma) diluted in 0.05 M PBS, pH 5.5–6.3, concentrations: 0.25–50 units/ml, at room temperature and at 4°C; (3) enzymatic digestion with the hyaluronidase from rat testes (Sigma) diluted in 0.02 M PBS, pH 6.8, concentration 50 units/ml, at room temperature and at 4°C; (4) conjugation with lectins: Concanavalin A from Jackbean (Calbiochem), and PHA type V from *Phaseolus vulgaris* (Sigma), concentrations 20–200 μg/ml, pH 7.2, at room temperature as advised by Kovacs and Csaba [3]. For transmission electron microscopy (EM),

cells were fixed in OsO_4 1% (5 min), followed by fixation in the mixture of 1% glutaraldehyde and 1% OsO_4 in 0.05 M cacodylate buffer for 1h, postixed in 1% OsO_4 for 1h, dehydrated in acetone and embedded in Epon. Sections stained with uranyl acetate and lead citrate were analyzed in EM TESLA.

1. EM sections of conditioned, untreated cells revealed many typical mucocysts inserted among the alveoles in the cortex of Tetrahymena (Fig. 1). 2. After AB test, about 95% of cells displayed extrusion of pseudocysts (Fig. 2). AB applied in vivo may be rediscovered in EM sections in pseudocysts wall and on the surface coat of cell membrane.

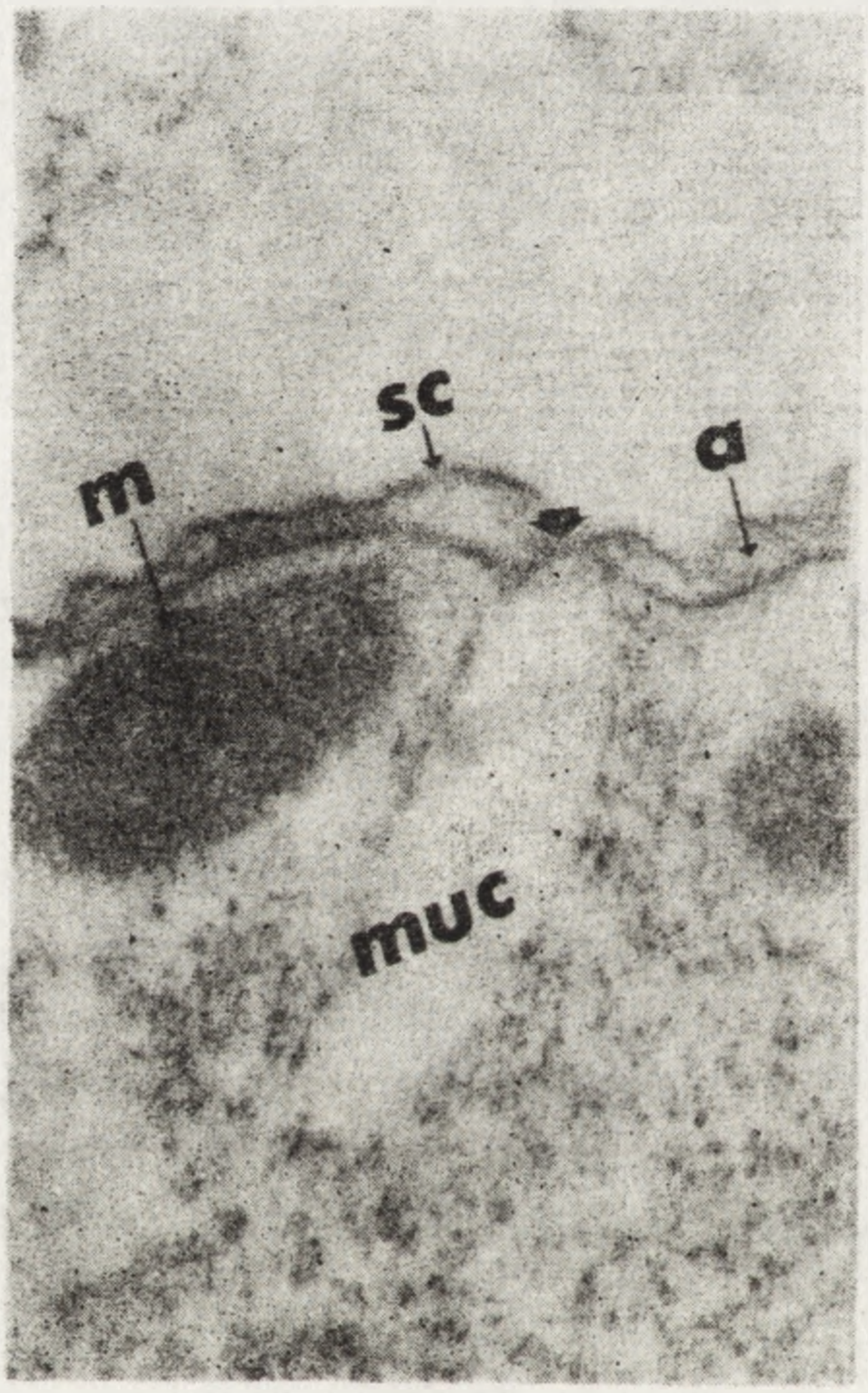

Fig. 1. The cortical area of untreated (control) *Tetrahymena thermophila*. The alveoles (a) are interrupted by the insertion sites (an arrowhead) of undischarged mucocysts (muc). Abbreviations: sc — surface coat, m — mitochondrium. Magn. 18 000 ×

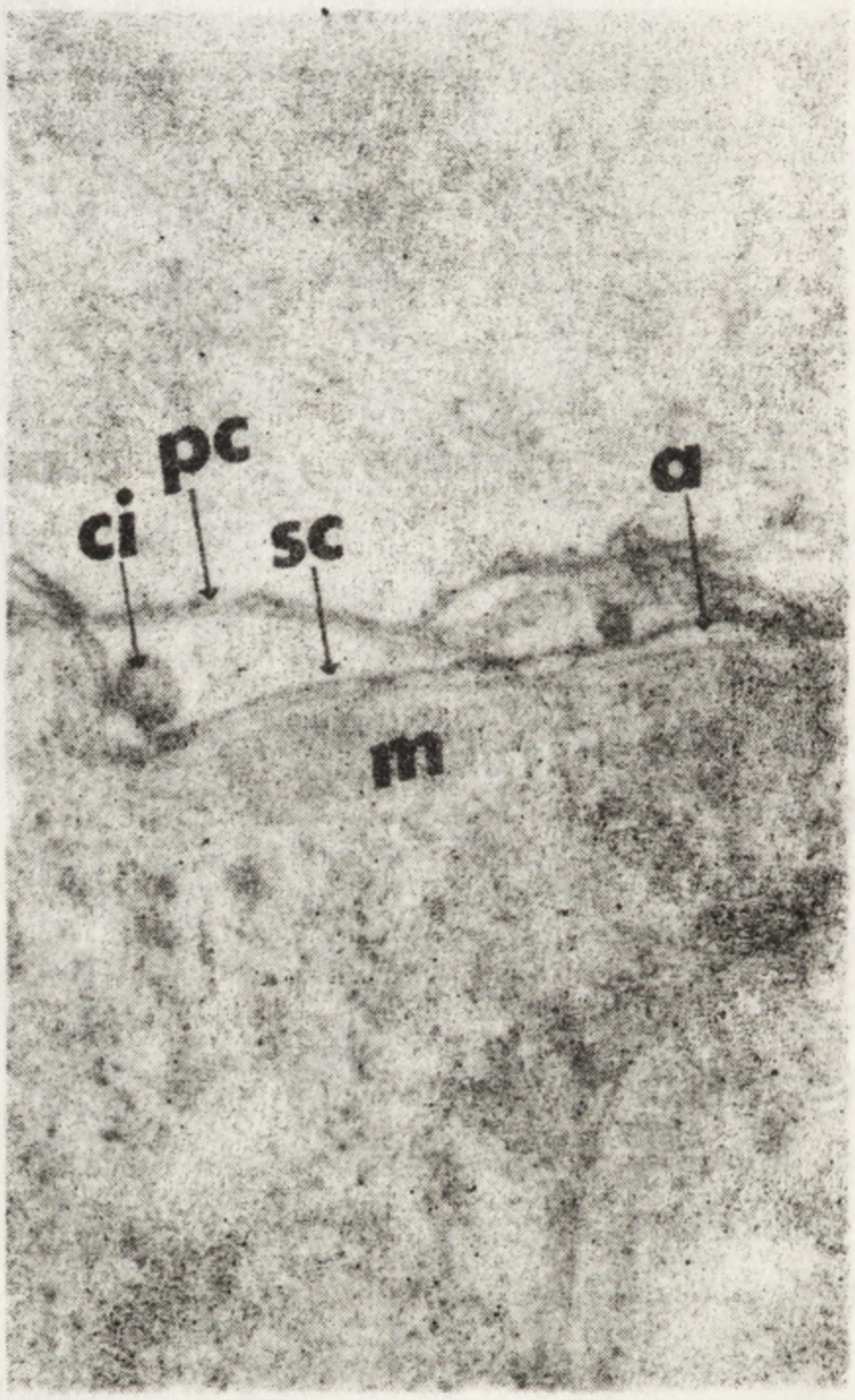

Fig. 2. The cortical area of *Tetrahymena* after AB stimulation. The pseudocysts (pc) surround whole cell including cilia (ci). Alveoles are flattened, there is no more mucocysts and mitochondria underline cortical area. Magn. 15 000 ×

3. Cells incubated with Mg^{++} before AB test demonstrated inhibition of the pseudocysts formation in about 85% of cells. In sectioned cells, the majority of mucocysts rested in situ, however, some of them were caught in statu nascendi of their bursts (Fig. 3). 4. Treatment of the

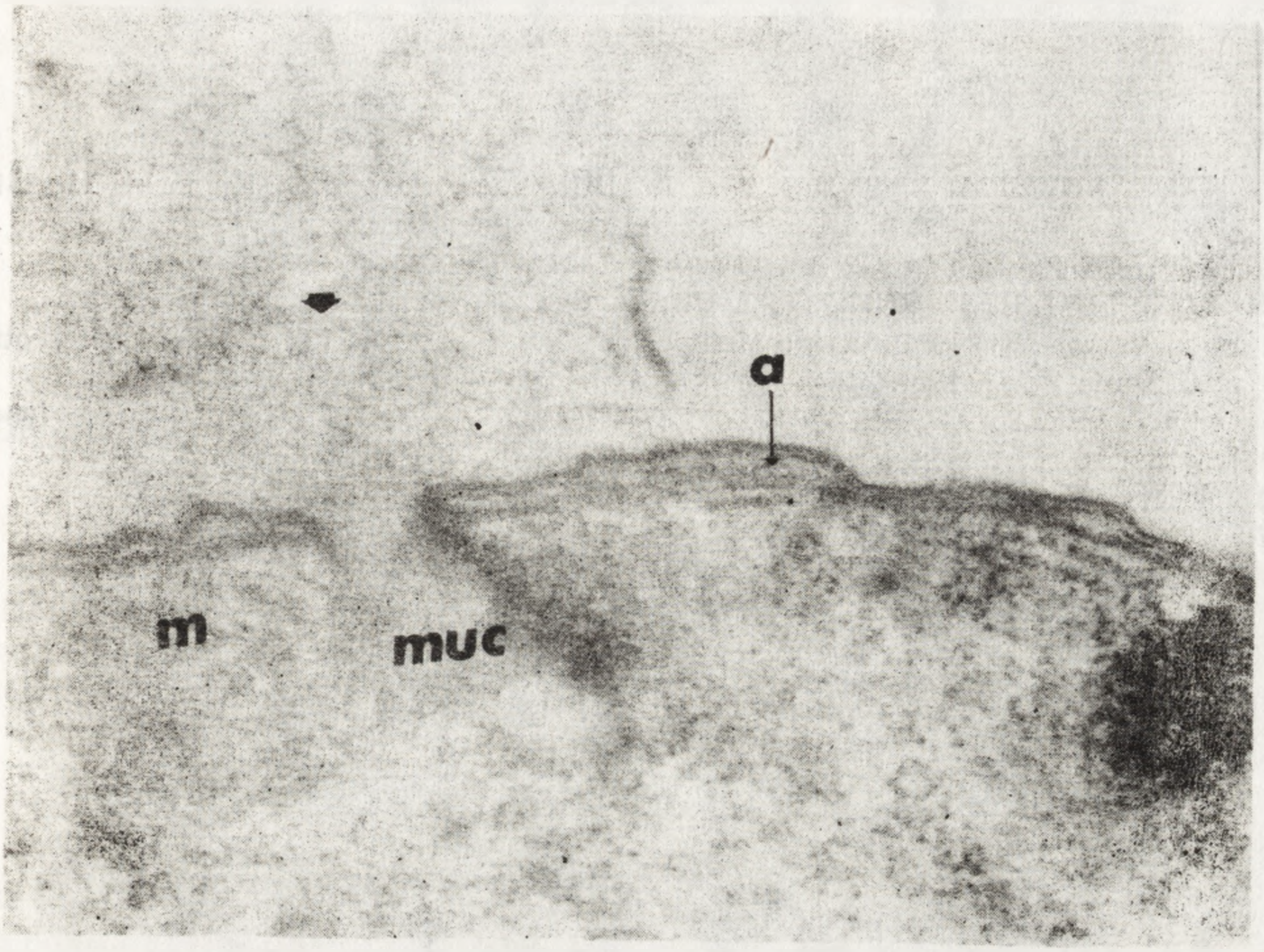

Fig. 3. Delayed discharge of one mucocyst (muc) in *Tetrahymena* preincubated with 15 mM $MgCl_2$ for 10 min, followed by AB stimulation. Most of the mucocysts remain in situ (unshown). An arrowhead — the mucilage extruded from the mucocyst. Magn. 48 000 ×

surface coat of *Tetrahymena* with both enzymes did not suppress the massive secretion of mucocysts in the AB tests. 5. Incubation of cells with lectins did not modify the AB stimulated secretion of mucocysts. Since concentration of Mg^{++} used here is below the critical electrolyte concentration (CEC) [6], Mg^{++} seems to act specifically. It is suggested that sialic and hyaluronate residues exposed in surface coat, and conjugation of active residues with lectins [3], are not involved in the mechanism of stimulus secretion coupling. It turns out that AB applied in vivo may be traced in EM sections.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3–4, 1984 (409–410)

UPTAKE AND METABOLISM OF LIPID INTERMEDIATES OF PROTEIN GLYCOSYLATION BY ANIMAL CELLS CULTURED IN VITRO

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Dolichol phosphates act as intermediates in glycosylation processes in Eucaryotic organisms. The role of relatively large amounts of unphosphorylated dolichols and the occurrence of fully unsaturated polyprenols in animal tissues, are not elucidated. Primary chicken embryo

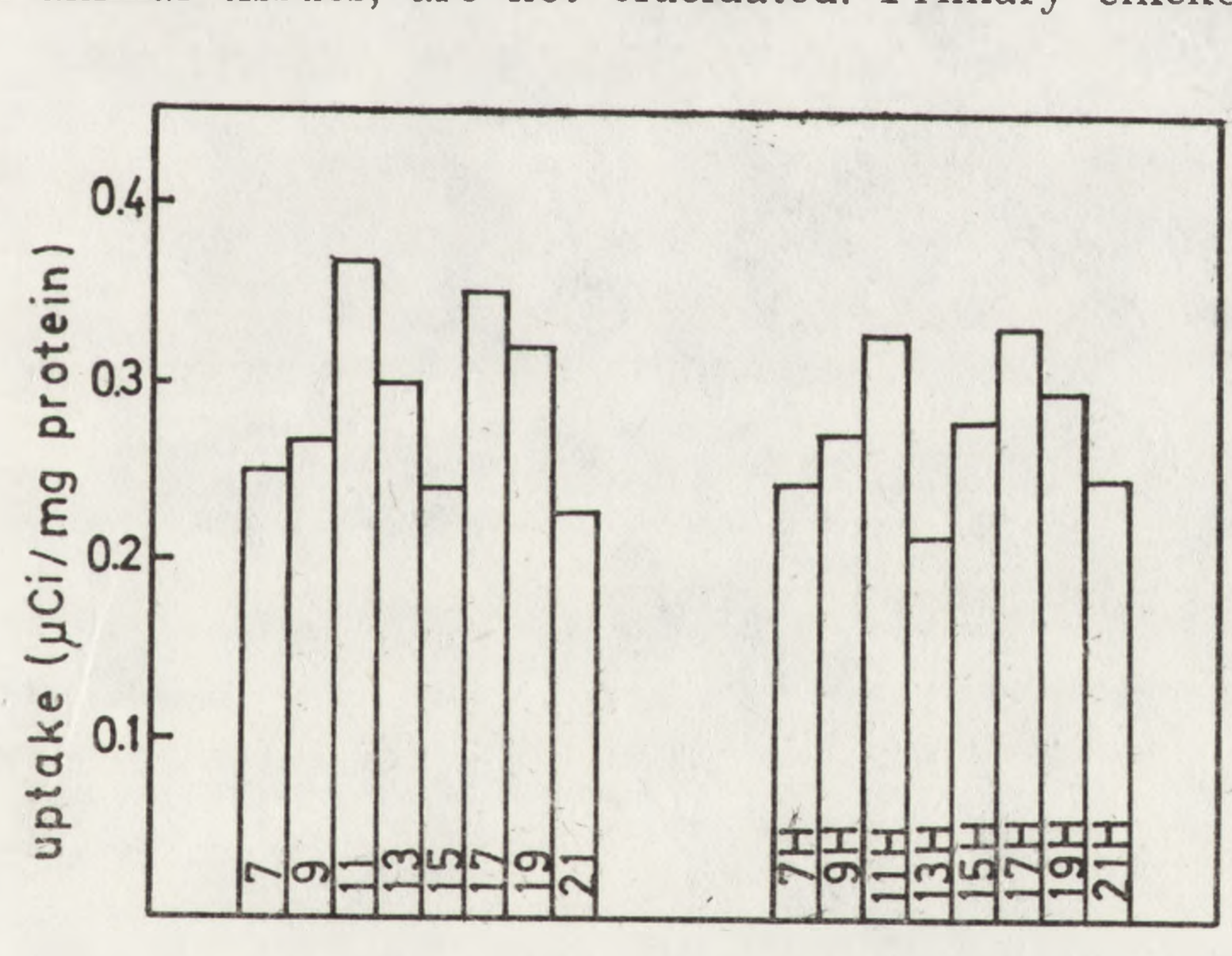

Fig. 1. The uptake of various polyprenols by primary cultures of chicken embryo fibroblasts. ^{3}H-labelled polyprenols (15 μCi) were supplied to monolayer cultures. After 20 h incubation, cells were extracted with chloroform: methanol mixture, 2 : 1 v/v. The uptake is expressed as total radioactivity of lipid extract per mg of protein. Symbols in the bars describe the type of polyprenol (number, number of isoprene residues; H, presence of saturated α-isoprene residue)

fibroblast cultures were used for studying uptake and metabolism of various types of polyprenols. Fully unsaturated polyprenols, α-saturated polyprenols (dolichols) and dolichol phosphates tritiated at C–1, were prepared and supplied to the incubation medium. The chicken embryo fibroblasts were found to take up all investigated polyprenols. The highest uptake was observed with prenologues C_{55} and C_{85}, and the lowest with prenologues C_{35}, C_{65}, C_{75} and C_{105}. After 20 h incubation most of polyprenols taken up were unchanged, and the major metabolic products were their esters with fatty acids. A small but detectable amount of incorporated polyprenols was modified by phosporylation and by substitution with carbohydrate groups. Fully unsaturated polyprenols were converted into the α-dihydropolyprenols. This conversion was observed mainly in the fraction of fatty acid esters. The uptake of dolichol phosphates was about 10 times lower than that of free alcohols. The highest uptake was observed in the case of C_{35}, C_{65}, C_{75} and C_{105}-dolichol phosphates.

POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (411—412)

THE EFFECT OF ELECTROMAGNETIC FIELDS (INDUSTRIAL FREQUENCY 50 Hz) ON PERMEABILITY OF BIOLOGICAL MEMBRANES IN THE PROCESS OF TRANSPORT AND ACCUMULATION OF IONS

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The results obtained in the research on plant organisms (cell, tissue, organ) demonstrate that the electromagnetic field (50 Hz) in reality considerably affects the growth and development of research plants. The intensive growth of research plant material in definite values of intensity of external electromagnetic field, was followed by the increase in receiving and accumulation of fundamental chemical elements (K, Na, Ca, P, Fe). The research with callus tissue of carrot and meristematic tissue of orchids (Cymbidium Lib. 67/2), confirmed that external electromagnetic field (frequency 50 Hz) of intensity in interval values 38–40 kV/m increased the accumulation of fundamental mineral elements in the tissue under study by about 20% in relation to control sample [3]. Also research of animal nervous preparation proved the influence of electromagnetic fields (50 Hz) on the change of electrophysiological parameters of nerves connected with the changing of transport of ions K^+ and Na^+ through biological membranes of nervous cells [1]. The obtained experimental results required creating the probable theoretical model of the interaction between electromagnetic field and fundamental structure of living cell (i.e. biological membrane). Using the existing "sensor-field" mode [1] in membrane channel, conjugated the transport of ions in the membrane, and was connected in the presence of external electromagnetic field (50 Hz) with conformation changes of channel caused by "field-sensor" (Fig. 1). The interaction between the electromagnetic external field (the intensity of field) and membrane channel with "field-sensor" (the change of

conformation), determines the changeability of ion flow, in consequence resulting in the changing of accumulation [2]. The suggested measure of the change of accumulation is the ratio of the function of membrane channel conformation without and within external field.

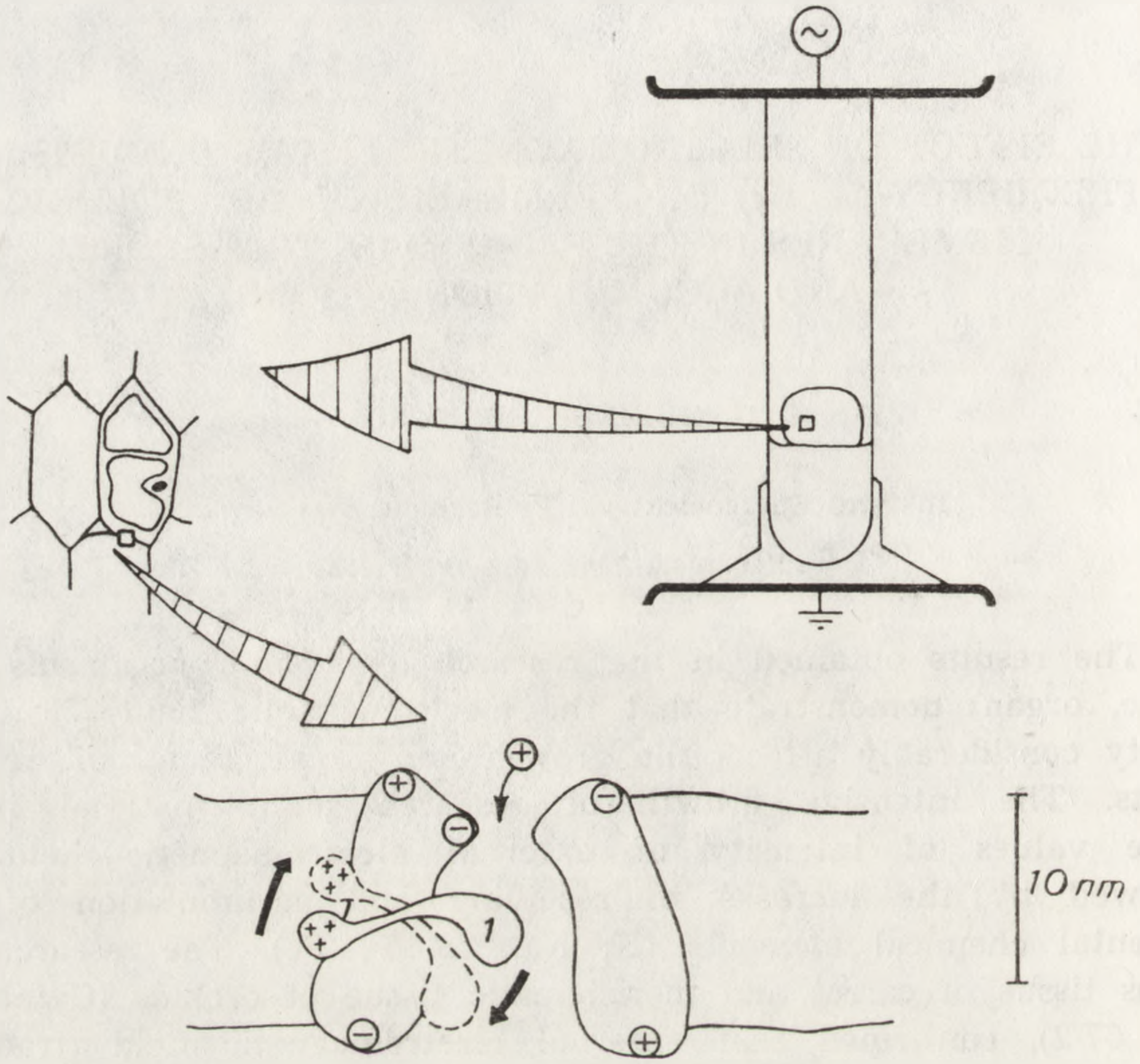

Fig. 1. The effect of electromagnetic field (frequency 50 Hz) on the transport of ions through membrane channel with (*1*) field-sensor

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (413—416)

TRANSFORMATION, RECRYSTALLIZATION AND DECAY OF PROLAMELLAR BODIES IN ETIOLATED PEA SEEDLINGS IN WHITE, RED AND BLUE LOW INTENSITY LIGHT

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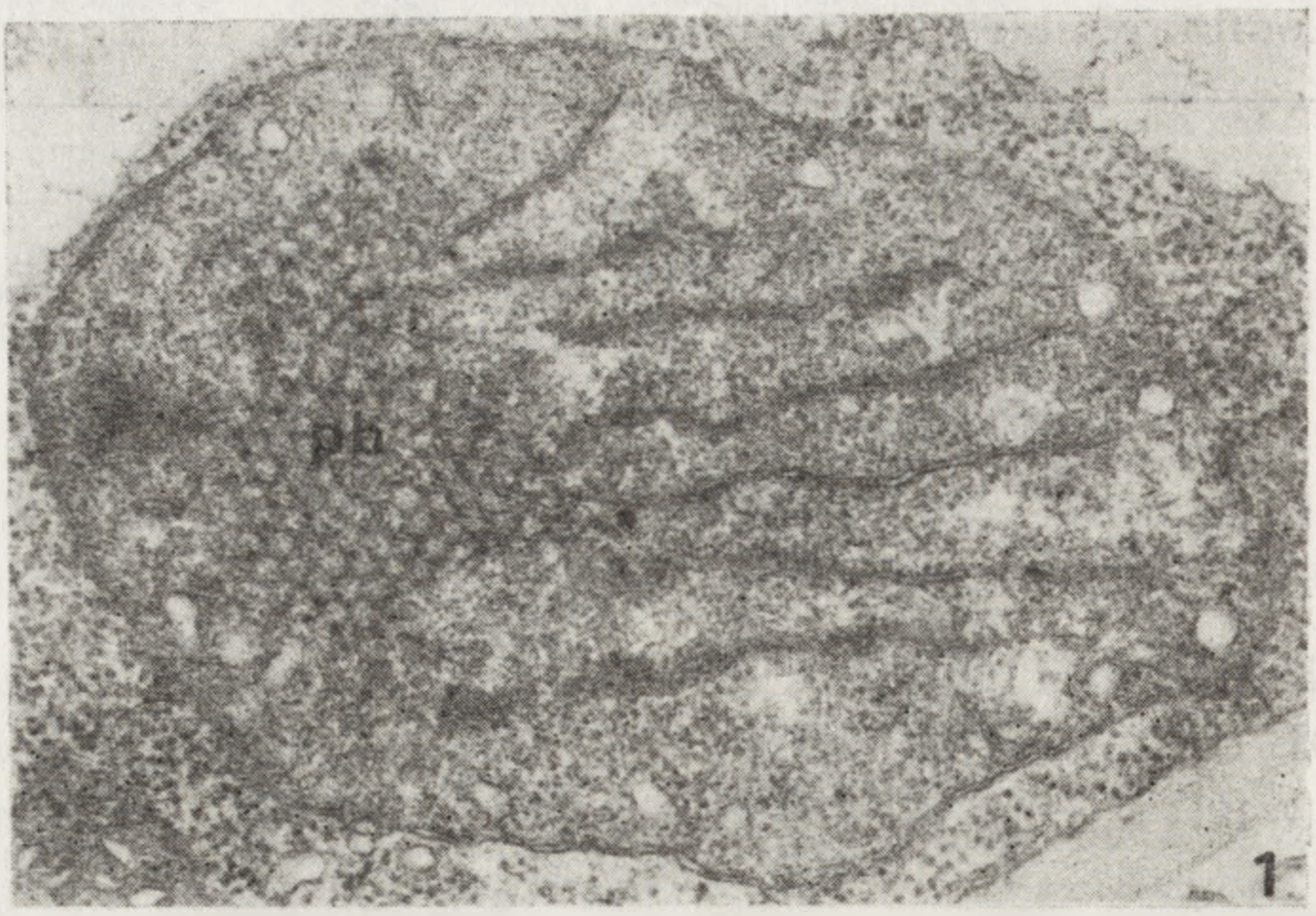
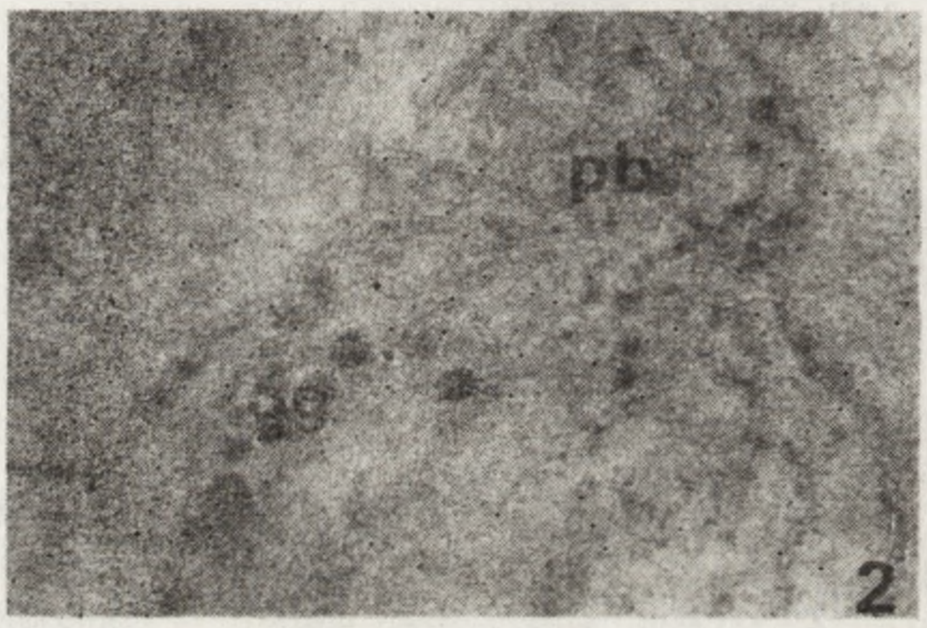
In this work a detailed study of prolamellar body reaction to blue, white and red low intensity continuous light was undertaken to elucidate the role of the wavelength of light in plastid development.

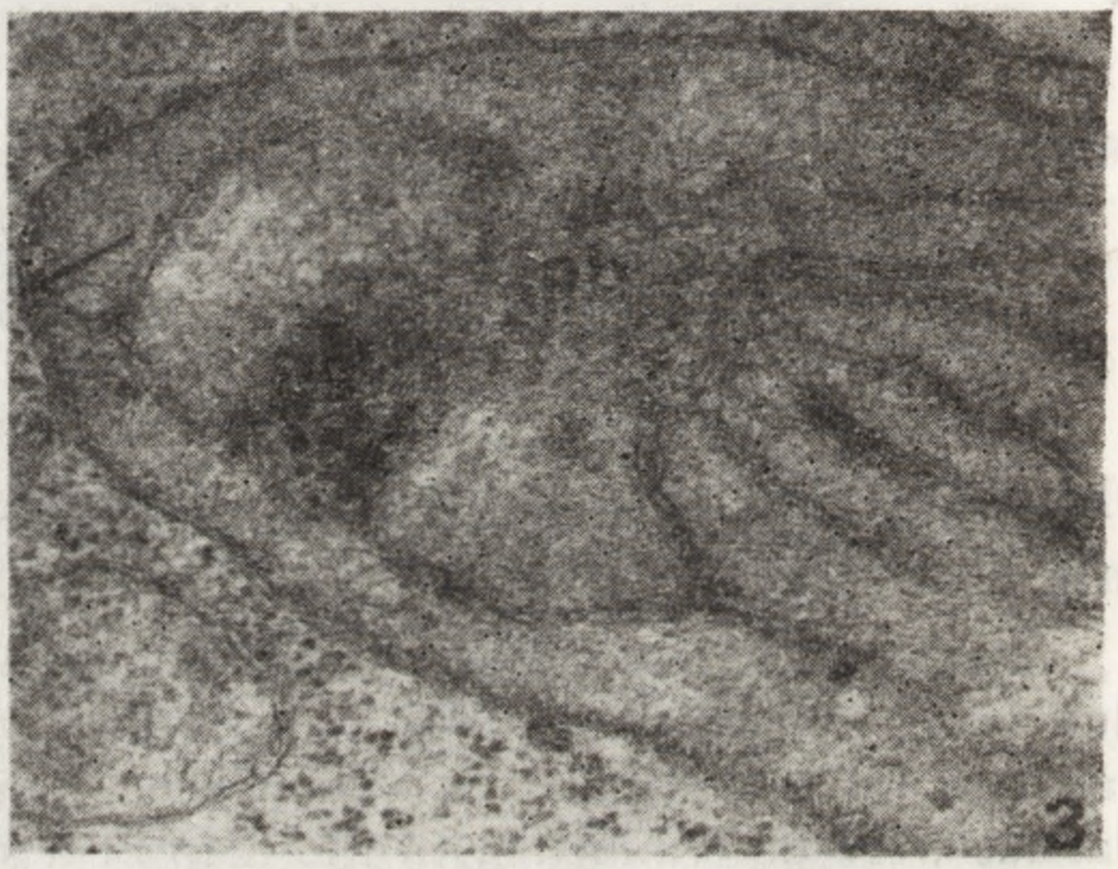
8-day-old dark-grown pea seedlings were illuminated by white, red and blue low intensity light over extended periods of time. The number of photons was the same in all cases. Dimensions and number of prolamellar bodies in plastids were determined with the help of a stereometric analysis.

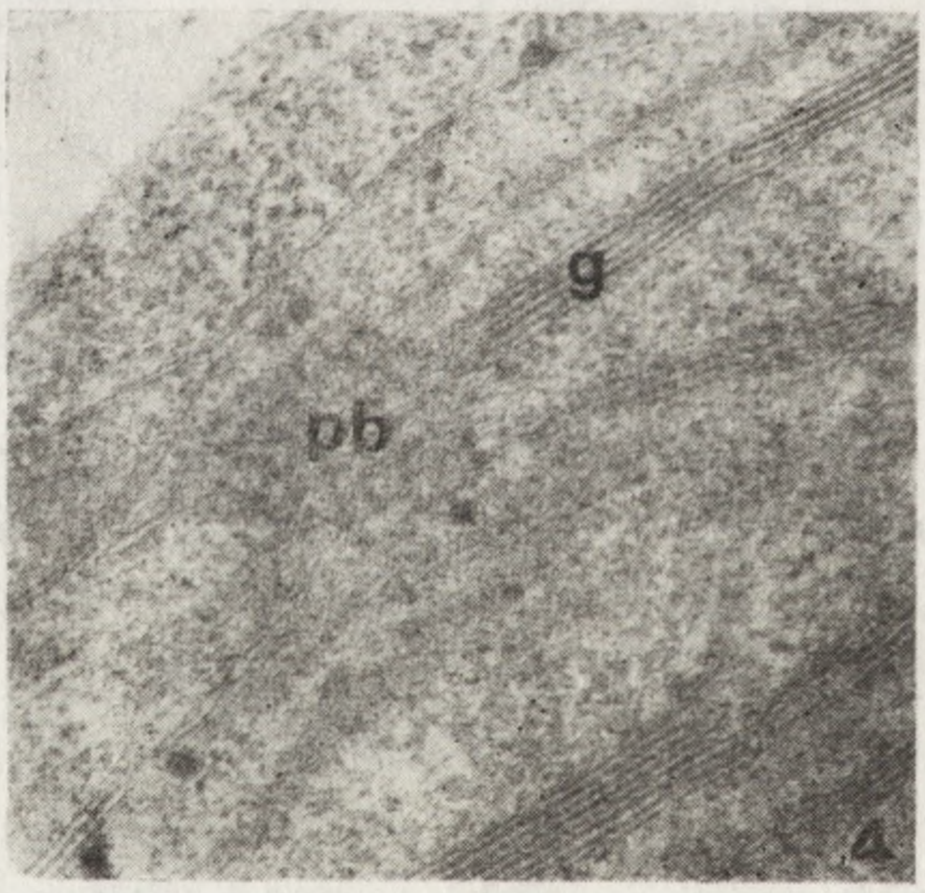
Etioplasts had on the average one paracrystalline prolamellar body with dimensions ranging from 0.6 to 1.6 μm in diameter (Fig. 1). After 2 hours of illumination some prolamellar bodies were transformed. The largest number of plastids with transformed prolamellar bodies (78%) was found in red light treatment, less in white (66%) and in blue (50%) lights (Figs. 2 and 3). The dimensions of prolamellar bodies were significantly reduced during transformation. After 2 hours of illumination the total average volume of prolamellar bodies was reduced by the largest factor (from 0.72 μm^3 in etioplasts to 0.19 μm^3) in white light. Accordingly, the number of prolamellar bodies increased by the largest factor in white light treatment (up to 3.46 on average). Reduction of prolamellar body dimension was due to dispersion and division into smaller bodies. The existence of the osmiophilic globules was also evidence of the prolamellar body dispersion.

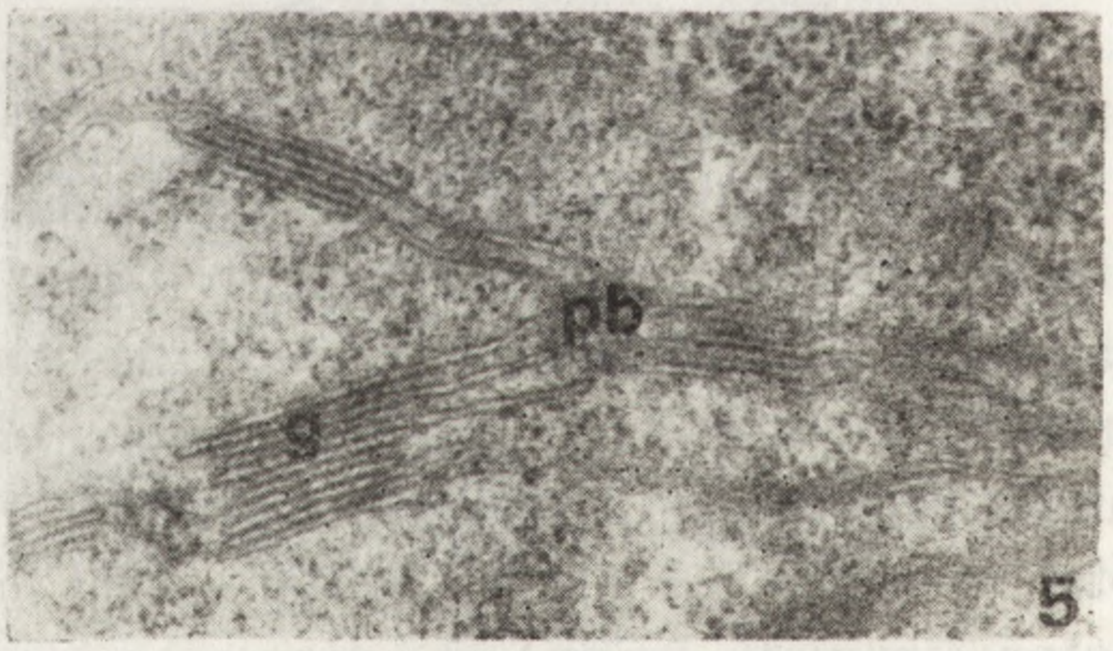
Recrystallization started after 4 hours of light treatment. In blue light 88% of plastids contained paracrystalline prolamellar bodies, 82% in white and 67% in red light 4-hour-long treatment. Paracrystalline prolamellar bodies were either formed during the recrystallization process, or originated from the division of bigger prolamellar bodies,











pb — prolamellar body, og — osmiophilic globule, g — granum

Fig. 1. Etioplast of 8-day-old dark-grown seedling

Fig. 2. Reacted prolamellar body after 2 hours of red light illumination. Porous peripheral lamellae indicated by arrow

Fig. 3. Paracrystalline prolamellar body after 2 hours of white light illumination. Invagination of membrane from the inner component of plastid envelope indicated by arrow

Fig. 4. Paracrystalline prolamellar body after 48 hours of red light illumination

Fig. 5. Rudimentary prolamellar body after 48 hours of blue light illumination

All figures × 64 000.

or did not at all undergo transformation. Prolamellar bodies formed in the dark and in low intensity light during the recrystallization, were morphologically the same. The decrease of the prolamellar body dimensions between 2 and 4 hours of illumination was now the largest in red light (from 0.38 μm^3 to 0.12 μm^3). Accordingly, the average number of prolamellar bodies increased by the largest factor of 3.5 in red light, and reached 8.61 per plastid.

The number of plastids with paracrystalline prolamellar bodies decreased further after longer red and blue light treatment, probably due to transformation of a small number of prolamellar bodies. The total volume of prolamellar bodies rapidly decreased between 4th and 12th hour of blue light down to 0.06 μm^3 per plastid. Further recrystallization of prolamellar bodies was observed after 24 hours of blue, white and red light treatment. All plastids had paracrystalline prolamellar bodies after 24 hours of blue light treatment. Recrystallization was completed after 48 hours in white light, and almost completed after 72 hours of red light treatment.

In the blue light the division and dispersion of prolamellar bodies led to rapid decrease of their volume (down to 0.004 μm^3 after 48 hours) and to their total disappearence after 72 hours of blue light treatment (Fig. 5). On the contrary, the total volume of prolamellar bodies increased to 0.34 and 0.32 μm^3 after 48 hours of red (Fig. 4) and white light respectively. Simultaneously, the number of prolamellar bodies per plastid slightly increased. Probably this was due to the formation of new small prolamellar bodies and their subsequent merging.

Summarizing, red and white light had the strongest influence on the prolamellar bodies in the initial stage of light treatment. This was manifested by the highest rate of transformation in red and white light treatment. The rate of the prolamellar body decay due to dispersion and division into smaller ones, is the largest initially (during the first 2 hours) in white, later (2–4 hours) in red and finally (after 4 hours) in blue light treatment. However, blue light turned out to be the most effective (during longer illumination) because of the fast and total prolamellar body decay.

White, blue and red light treatment led to transformation, recrystallization and decay of the prolamellar body. These processes were quite similar in all lights. However, the rate of these processes and the percentage of plastids in which these processes took place were different.

Chloroplast development in low intensity light treatment is different from that in normal conditions. Low intensity light leads to transformation and later to reformation of paracrystalline prolamellar bodies which exist in plastids simultaneously with grana.

POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3-4, 1984 (417-418)

STUDIES OF THE INTERACTION BETWEEN LIPOSOMES AND SOME GLYCINE ESTERS

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The interaction of liposomes with some glycine esters was investigated by titration calorimetry at 25°C. The values of enthalpy change of the interaction were measured. The CMC values were also measured. It is suggested that interaction of N,N,N-trimethylglycine chloride decyl ester (RW-315) with liposomes is of hydrophobic nature, whereas that of N,N,N-trimethylglycine chloride 2-chloroethyl ester (RW-19) is electrostatic in character.

It has been found in our laboratory that some glycine esters with biological activity have an influence on ion transport across the liposome membranes and on hemolysis of pig red blood cells. To elucidate these effects, calorimetric studies have been performed.

Experiments were carried out using home-made calorimeter. The reaction vessel contained 25 cm^3 buffered suspension of liposomes. The liposomes were formed from about 0.1 g egg yolk lecithin by sonification in 0.01 M Tris-HCl, pH 7.5 buffer solution. Buffered solutions of RW-19 and RW-315 were used as titrants. The concentration was about 0.1 mol/dm^3. The compounds were synthesized in the Institute of Organic and Polymer Technology (Technical University of Wrocław).

The studied RW-19 and RW-315 compounds are surface active substances. Thus knowledge of the CMC value was of great importance. The dye absorption method of CMC determination was applied. In case

of RW-315 compound the CMC value was 0.02 mol/dm^3. However in case of RW-19 compound the CMC value could not be determined. Since the concentration of RW-315 was above the CMC, the demicellization process preceded the interaction with liposomes. Thermal effects of demicellization were found and allowed for in final calculations. The enthalpy changes were calculated per mole of the RW-19 and RW-315 compounds. The following values were obtained: -7.3 kJ/mol RW-19 and -4.2 kJ/mol RW-315. The absolute value of the change is greater in the case of RW-19. According to our preliminary interpretation this can be explained by hydrophobic interaction in case RW-315 compound and electrostatic binding in case of RW-19. Further studies with homologous compounds of RW-315 compound are in progress.

POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (419—420)

MOMENTUM TRANSPORT IN OSMOSIS, THERMODIALYSIS AND THERMODIFFUSION

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Volume flow due to solute concentration gradient (osmosis) as well as that due to temperature gradient (thermodialysis) can occur without pressure gradient, and usually does occur against pressure gradient, i.e. from lower to higher pressure. This is possible in the case of osmosis owing to the interaction between the solute and membrane, the interaction resulting in momentum transport between the solution and the vessel to which the membrane is fixed [1–4]. In the case of thermodialysis and thermodiffusion, the momentum transport between the solid (membrane, solute particle) and liquid phase takes place due to variation in the components of the thermal conductivity tensor near the solid-liquid interface boundary. The proposed theory is based on the assumption (experimentally well justified [5–8]) that heat flux, $\bar{I}_q$, conducted through a medium carries momentum flux $\bar{I}_p = \frac{H}{u}\bar{I}_q$, where u is velocity of phononic thermal excitations and H is a numerical constant close to unity [7]. For anisotropic media the relationship has the form: $\hat{I}_p \cdot \bar{u} = H\bar{I}_q$, where $\hat{I}_p$ is the heat-carried momentum flux tensor and $\bar{I}_q = -\hat{k}\ \mathrm{grad}\ T$ with $\hat{k}$ — thermal conductivity. The momentum $\hat{I}_p$ must be allowed for in the momentum balance equation, which then has the form:

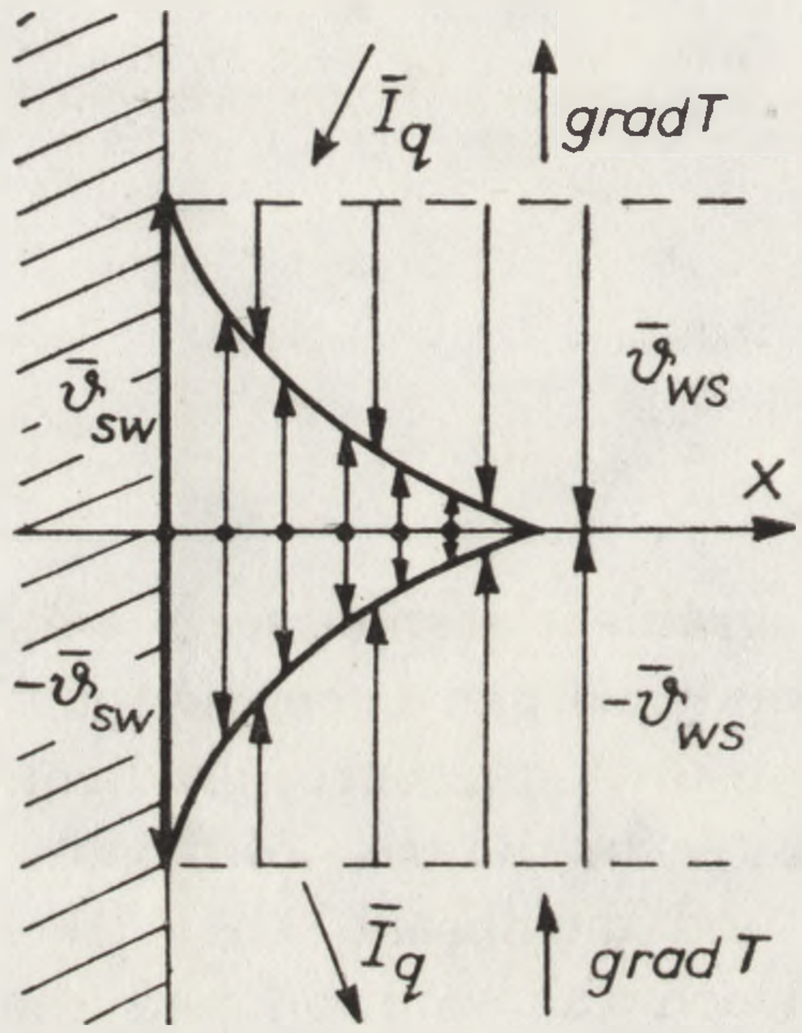
$$\frac{\partial}{\partial t}\left(\varrho\bar{v}\right) = -\mathrm{Div}\ (\varrho\bar{v}\bar{v} + \hat{P} - \hat{I}_p) + \sum_k \varrho_k \bar{f}_k . \qquad (1)$$

The pressure tensor for liquids can be written as: $\hat{P} = pU + \Pi^v U + \overset{\circ}{\Pi}_s$ and $\hat{I}_p = \frac{H}{u}\bar{I}_q\bar{n}$ is the dyadic product of $\bar{I}_q$ and $\bar{n} = \bar{u}/u$. Equation

(1) when applied to a simple case of flat interface (see the figure) and stationary state without external forces ($f_k = 0$) gives:

$$\eta \left.\frac{\partial v_y}{\partial x}\right|_o = \pm \left| \Delta \left(\frac{H}{u} k_{xy} \frac{dT}{dy} \cos \beta \right) \right|_\infty^o . \tag{2}$$

The absolute value of the expression on the right side of Eq. (2) is to be taken with (+) sign when the component k_{xy} of the thermal conductivity tensor decreases on approaching the solid phase surface and with (−) sign when the opposite takes place. Δ denotes that a difference of the expression between a point on the interface and a point well within the liquid phase should be taken. From equation (2) follows that a viscous flow of liquid near the interface can occur due to temperature gradient along that interface (as shown in the figure). The upper half of the picture depicts the situation when the momentum transmission coefficient, $\frac{H}{u} k_{xy}$ of the solid phase is larger than that of the liquid. The solid phase then moves with velocity $\bar{v}_{sw}$ relative to the liquid or the liquid flows with velocity $\bar{v}_{ws}$ relative to the solid when the latter is kept at rest. The lower part of the figure depicts the same for solid phase having smaller momentum transmission coefficient than the liquid. Experimental verification of the theory is given in papers [5, 6].



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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (421—424)

THE EFFECT OF PHOSPHATIDYLSERINE ON THE ACTIVITY AND FLUORESCENCE PROPERTIES OF 3-PHOSPHOGLYCERATE KINASE FROM BOVINE HEART

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The binding of tetrameric glycolytic enzymes — glyceraldehyde-3-phosphate dehydrogenase [1] and FDP-aldolase [2] to model phospholipid membranes results in decreasing their enzymatic activities. In both cases the inactivation was associated with the red shift and quenching of intrinsic protein fluorescence. The changes of the fluorescence parameters and the inactivation were interpreted as a result of conformational changes of the protein after binding to the phospholipid membrane [2]. An alternative explanation which should be taken into account is an interaction of individual phospholipid molecules with the active center, or allosteric effects. It is also unknown, whether phospholipids cause dissociation of the tetramers into inactive subunits. For these reasons it was interesting to examine the effect of phospholipids on the activity and fluorescence properties of a monomeric enzyme.

3-Phosphoglycerate kinase (E.C.2.7.2.3.) appears to be the only glycolytic enzyme which is not composed of subunits [3]. It catalyses the reversible transfer of a phosphoryl group from $Mg^{-}ATP^{2-}$ to 3-phosphoglycerate [4]. The enzyme binds reversibly to erythrocyte membranes

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Abbreviations: PGK — 3-phosphoglycerate kinase,
PS — phosphatidylserine.

and it can be eluted from them in the presence of ATP or by incubation with 0.15 M NaCl [5]. The above results suggest electrostatic interaction of the enzyme with the negative charged membranes, although it is unknown whether it binds to phospholipid or protein component of the membrane.

Phospholipid liposomes were obtained either from bovine brain phosphatidylserine ("Koch-Light") or from synthetic dipalmitoyl phosphatidylserine ("Serva") by sonification of the lipid in 10 mM Tris-HCl buffer pH 7.6, over a time of 20 and 40 minutes, respectively, using MSE 150 W sonifier. Different amounts of such prepared suspension (PS concentration about 2.4×10^{-3} M) were added to a solution of PGK in the same buffer and enzymatic activity was determined according to Bücher [4]. The results are presented in Tab. 1. as a per cent of initial activity of the enzyme, which was 480 U/mg.

TABLE 1

The effect of natural and synthetic phosphatidylserine liposomes on the activity of PGK

	Lipid-protein molar ratio	% of initial activity	% of initial activity after 0,5 h of incubation
PGK alone	—	100	57
natural PS	13	115	100
	26	107	95
	50	55	—
	78	54	—
	115	26	—
synthetic PS	11	100	100
	22	100	100
	40	100	100
	67	75	75
	90	75	75
	110	68	68

At low lipid-protein molar ratios (10–40) full enzymatic activities were observed in the presence of both, natural and synthetic PS. In the former case intrinsic protein fluorescence of PGK was quenched with no shift in emission maximum, whereas synthetic PS did not change fluorescence parameters of the protein (Fig. 1). At higher lipid-protein molar ratios ($\sim$ 60–115) the enzyme loses about 50–75% of its initial activity in the presence of natural PS, whereas synthetic PS causes only 25–30% inactivation. Another effect observed at a low lipid concentration is stabilization of the enzymatic activity in diluted protein solution. Specific activities determined after about 0.5 hour incubation

of the diluted PGK solutions at room temperature are unchanged or insignificantly higher from the initial ones in the presence of PS, whereas they drop to about 60–70% in absence of PS. These results suggest that phospholipid membrane protects the enzyme against thermal in-

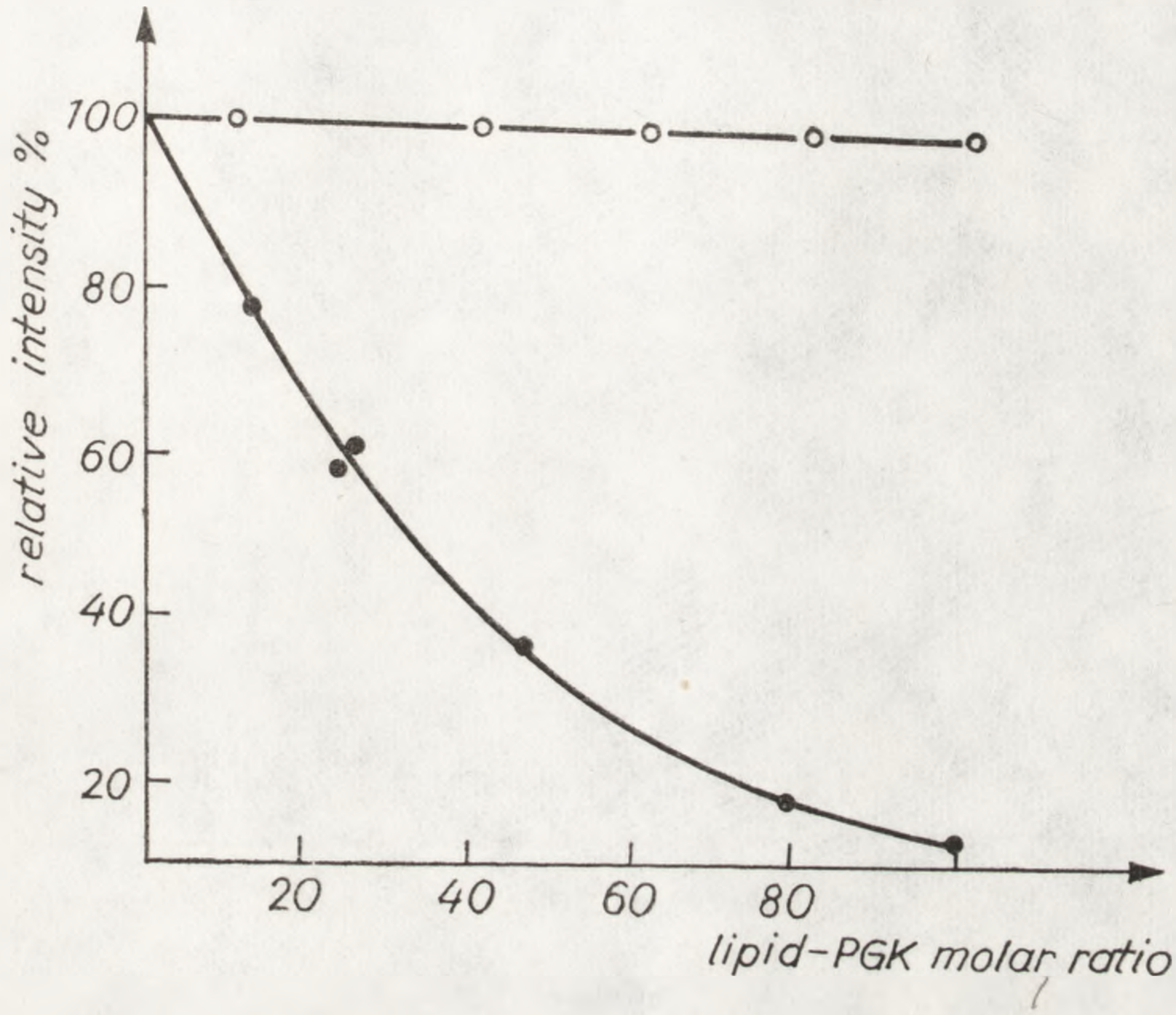

Fig. 1. The effect of natural (●) and synthetic PS on the intrinsic fluorescence of PGK. Synthetic PS — open symbols

activation, which takes place at experimental condition applied (20°C, protein concentration about 0.25 μM). It seems, that the fluorescence quenching and strong inactivation of PGK in the presence of natural PS is caused mainly by impurities of the phospholipid, whereas the protection of the enzyme against the inactivation is a result of its adsorption on the phospholipid membrane.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (425—428)

THE EFFECT OF PHOSPHATIDYLSERINE VESICLES ON CONFORMATIONAL CHANGES OF TRYPSIN LABELED WITH o-PHTHALDIALDEHYDE

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Primary amino groups of amino acids and proteins react with o-phthaldialdehyde (OPA) in the presence of 2-mercaptoethanol to yield highly fluorescent isoindole derivatives [1–3]. Their fluorescence parameters were found to be sensitive to the polarity of the environment [4], therefore it was attractive to use the isoindole fluorophore as a fluorescent probe in the studies of protein conformation [3]. Since absorption spectrum of the isoindole moiety overlaps with the emission spectra of protein tryptophans, resonance energy transfer may exist between these fluorophores. We have applied OPA to label trypsin, in order to show conformational changes of the protein, associated with its binding to phospholipid membranes. The labeling reaction was performed in borate buffer, pH 8, by the addition of OPA reagent prepared according to Roth [1]. At a sufficiently low degree of the labeling (OPA — trypsin molar ratio was 1.25) only external, water exposed amino groups of the protein are able to react with OPA and the reaction product is also water exposed (λ_{em} = 450 nm). Introduction of the probe to the protein molecule results in appearing of an additional maximum in its excitation spectrum (Fig. 1, band near 290 nm), dramatic decrease of the protein emission and sensitized fluorescence of the probe (Fig. 2, curve *1*). All these effects indicate the existence of resonance energy transfer between protein tryptophan, acting as energy donor, and the isoindole acceptor.

Suspension of phosphatidylserine (PS) was prepared from synthetic phospholipid ("Serva") by sonication in 12 mM sodium borate buffer, pH 8. Addition of the suspension to the labeled trypsin solution induces

the following changes in the fluorescence of both, protein tryptophans and the probe:

— an increase and red shift of the protein fluorescence,
— quenching and blue shift of the probe fluorescence (Fig. 2, curves *2* and *3*),
— an increase in polarisation degree of the probe.

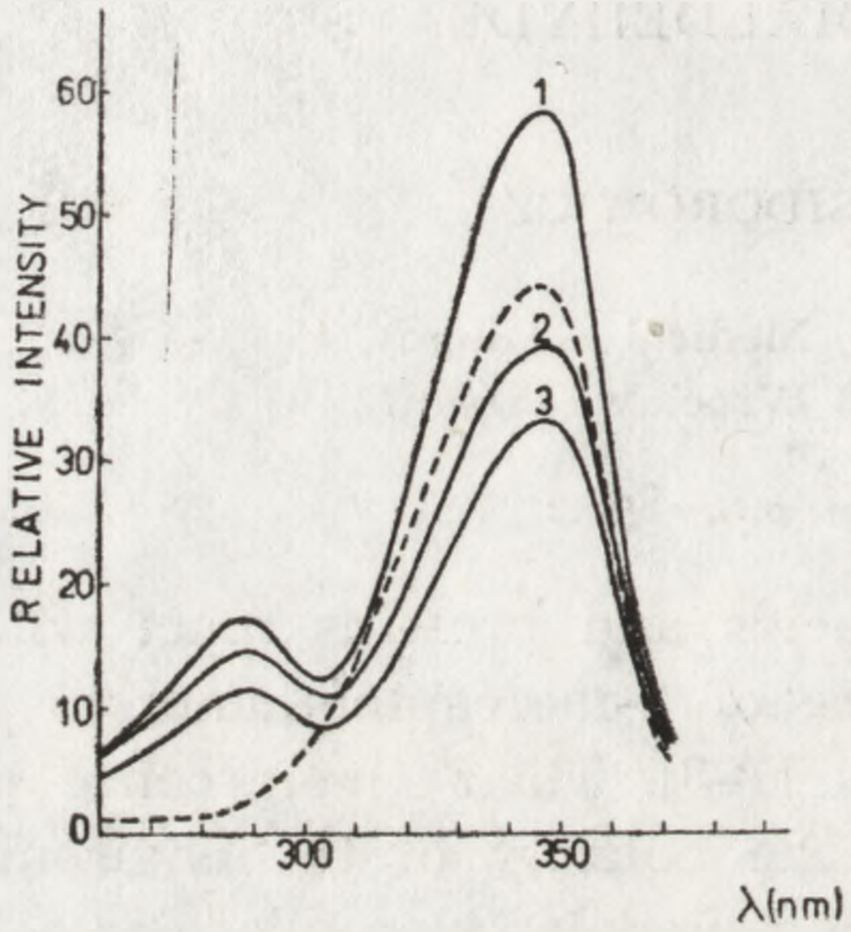

Fig. 1. Excitation spectra of OPA labeled trypsin in borate buffer, pH 8 (curve *1*) and after addition of 0.2 and 0.4 mM PS (curves *2* and *3* respectively). Trypsin concentration: 10 μM. The dashed curve represents the excitation spectrum of OPA labeled glycine. Emission monitored at 450 nm

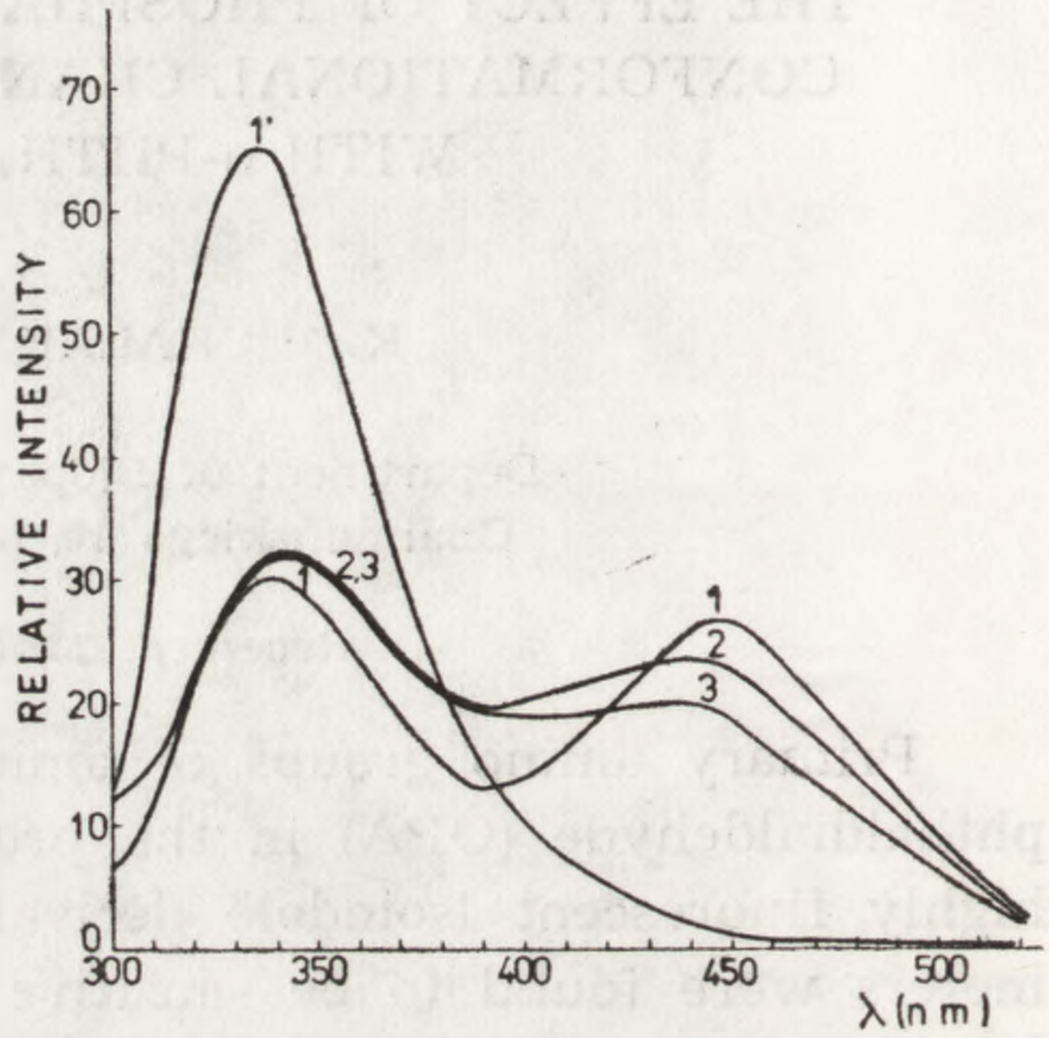

Fig. 2. Emission spectra of OPA labeled trypsin in borate buffer, pH 8 (curve *1*) and in the presence of 0.2 and 0.4 mM PS (curves *2* and *3*, respectively). Curve *1'* — the emission spectrum of unlabeled trypsin $\lambda_{ex} = 290$ nm. Protein concentration: 10 μM

The quenching of the probe and the simultaneous increase of the protein fluorescence can be explained as a result of an increase of donor — acceptor distance, which lowers energy transfer efficiency. This effect and shifts of the emmision maxima (337 to 348 nm for the protein fluorescence and 450 to 442 nm for the probe fluorescence) appear to reflect conformational changes of the enzyme bound to the phospholipid vesicles. It is consistent with the observations of Vaz et al. [5] concerning partial defolding of trypsin bound to negatively charged phospholipid liposomes. An increase in polarisation degree of the isoindole probe upon addition of PS suspension, which raised from 0.14 to 0.20, may result from an increase in microviscosity of the probe environment after binding to the phospholipid membrane.

The results presented above indicate that introduction of the isoindole probe into protein macromolecules can provide valuable information about protein conformational changes induced by ligand binding.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (429—432)

FORMING OF MYELIN-LIKE STRUCTURES IN THE EPITHELIAL CELLS OF RESIN DUCTS

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Forming of myelin-like structures in epithelial cells of resin ducts of *Picea abies* (L.) Karst. was observed in electron microscope. It seems that these structures correspond both with multilamellar bodies [4] and with periplasmic membrane vesicles [3] and with lomasomes [2]. This heterogenous terminology which we found in literature results from the fact that myelin-like structures are found in different types of cells in numerous plants and their full function and origin has not yet been described. Some authors think that these bodies are artifacts, but most of them still connect the occurrence of myelin-like structures in cells with autolitic processes [1].

Fig. 1. The epithelial cell of two-year-old shoot of *Picea abies*. 10 000 ×

Myelin-like structures in resin ducts of *Picea abies* were observed after double fixation of shoots in glutaraldehyde and OsO_4. Usually, they were composed of strongly osmophilic and closely adjoining to each other lamellae. Myelin-like structures in epithelial cells from one-year-old normal shoots did not occur or were found very rarely, however they were observed often in epithelial cells of older shoots (Fig. 1). These studies confirmed that myelin-like structures appeared very often in cells of resin ducts deformed by the aphis, *Sacchiphantes viridis* Ratz. The process of forming myelin-like structures is shown in Figs. 2 and 3 which are illustrating two terminal stages of differentiation of

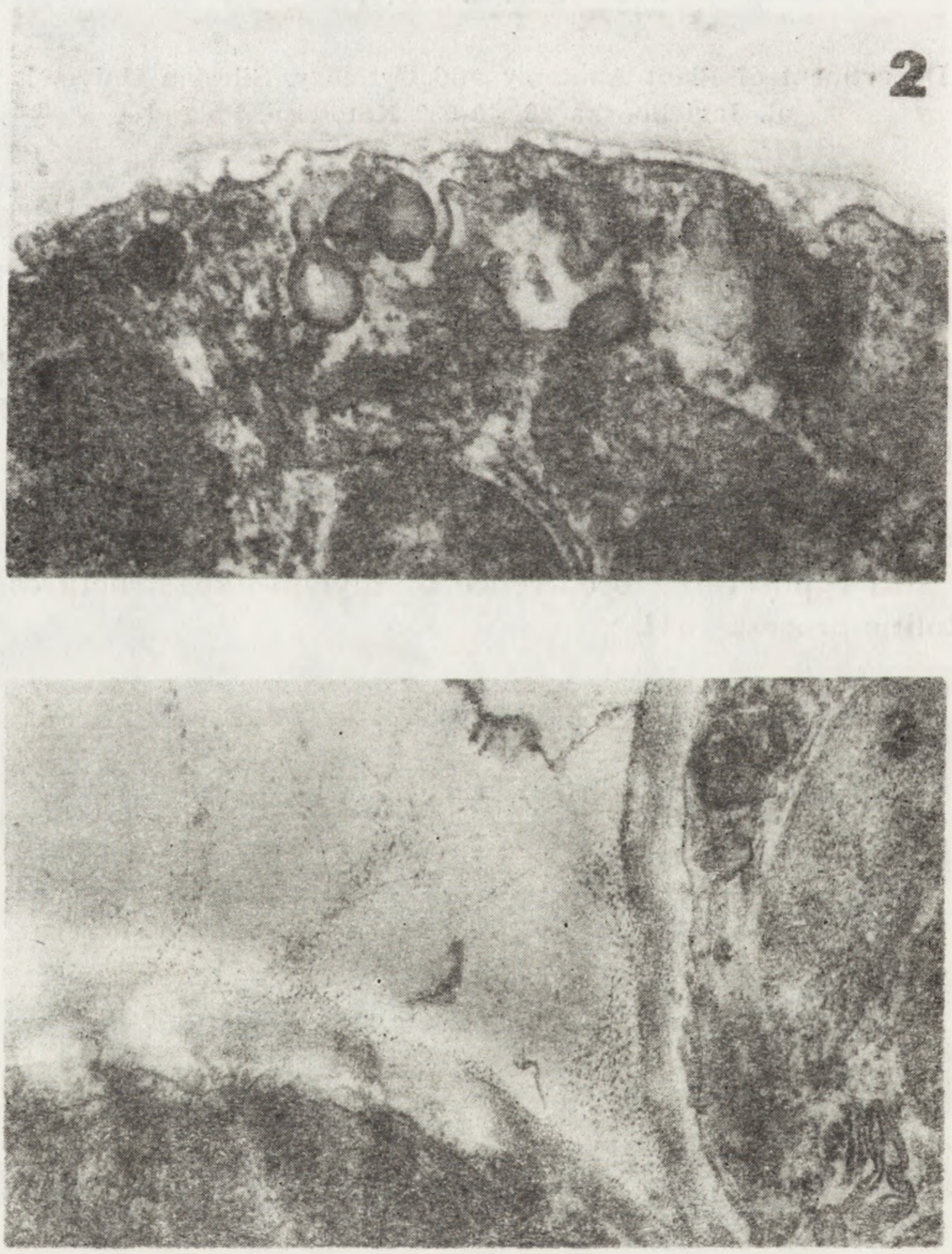

Fig. 2 and 3. The epithelial cells of one-year-old shoots of *Picea abies* deformed by *Sacchiphantes viridis*. 14 000 ×

epithelial cells in resin ducts of spruce deformed by *S. viridis*. The vesicles which contain terpenic material and derive from the endoplasmic reticulum, at first adhere to the plasmalemma (Fig. 2), then, they are set off into the periplasmic space. The terpenic material from the vesicles situated near the plasmalemma is accumulated in the form of lamellar structures (Fig. 3). Activity of cells secretion was considerably reduced at this stage. It seems that the myelin-like structures in resin ducts are formed in this way in all Conifers. In addition, it suggest that monoterpenes and sesquiterpenes are integral components of theirs strucures only when are transformed into complex forms of terpenes.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (433—436)

PARTICIPATION OF MEMBRANES OF GRANULAR VESICLES OF EPINEPHROCYTES IN GUINEA PIG, IN PROCESSES OF ADRENALINE SECRETION

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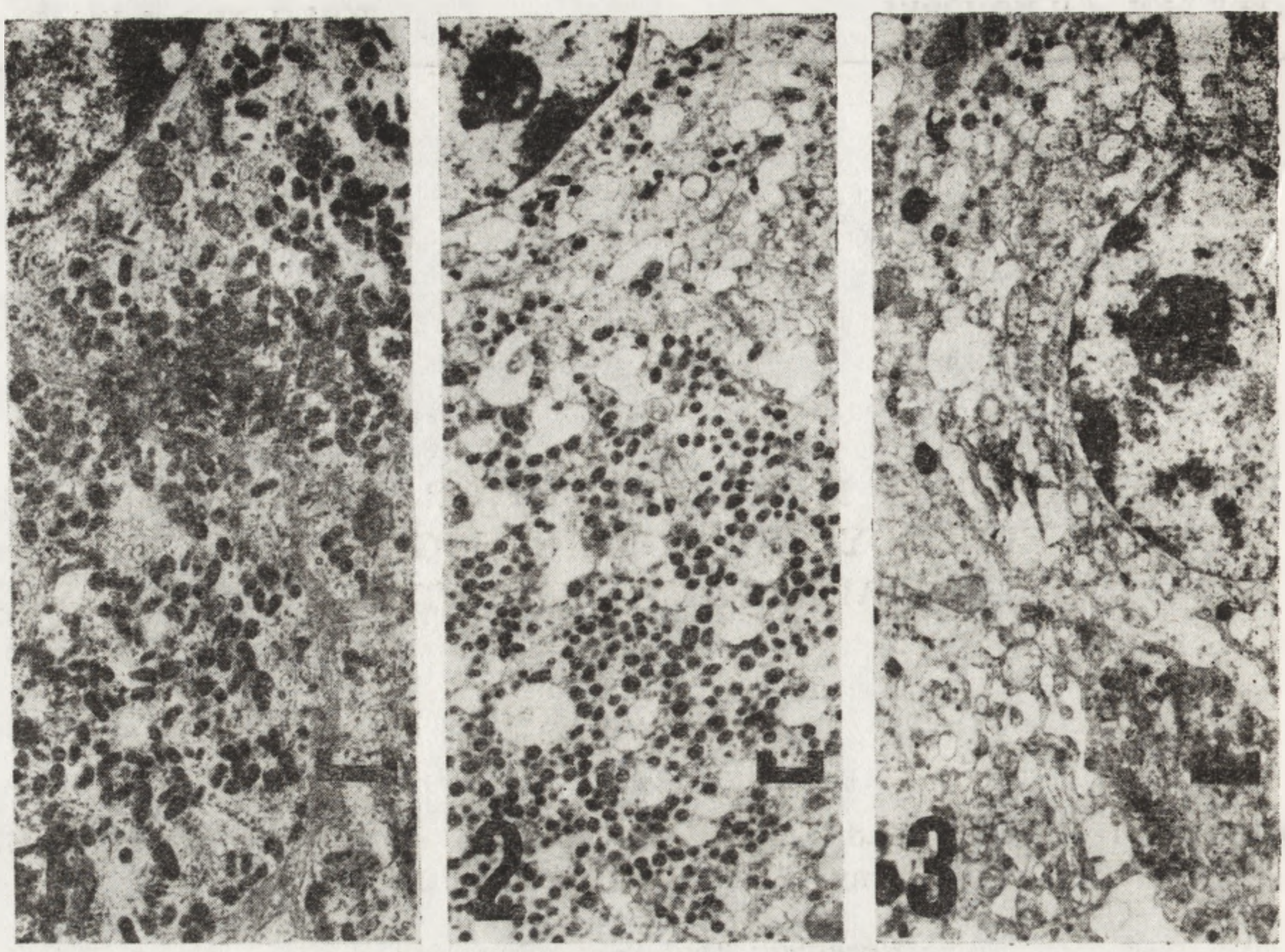
Many physio- and morphobiochemical studies [1–8] were devoted to secretion of catecholamines from cells of the adrenal medulla. In spite of very numerous data obtained, the problem is still far from being finally solved. In the course of the acute anaphylactic shock in the guinea pig, large amounts of adrenalin, among other substances, are secreted to blood [2, 3]. Histamine is the main mediator of this shock. It was also found that administering histamine to animals results in the release of adrenaline to blood circulation, dependent on the dose [3]. Thus, it seemed justified to follow ultrastructural changes in granular vesicles in the acute anaphylactic shock and after administering exogenous histamine in the so-called model of the lethal histamine shock, in order to observe processes of secreting adrenaline from epinephrocytes of these animals.

The study was performed on the adrenal medullae of 15 guinea pigs of body weight of 330 g in three groups of these animals, i.e. (1) in healthy guinea pigs, (2) in guinea pigs which had died in the acute anaphylactic shock caused by inhaling, after the earlier sensitization of animals with ovoalbumine, and (3) in guinea pigs which had died in the shock after administering histamine directly intracardially (0.75 mg/kg of body weight). Specimens of the adrenal medulla were fixed in Karnovsky and Dalton's fluids [3], embedded in Epon-812 and cut in ultrathin sections on the ultramicrotome LKB III. They were contrasted with uranyl acetate and lead citrate. Electronograms were made in Philips EM-300 electron microscope.



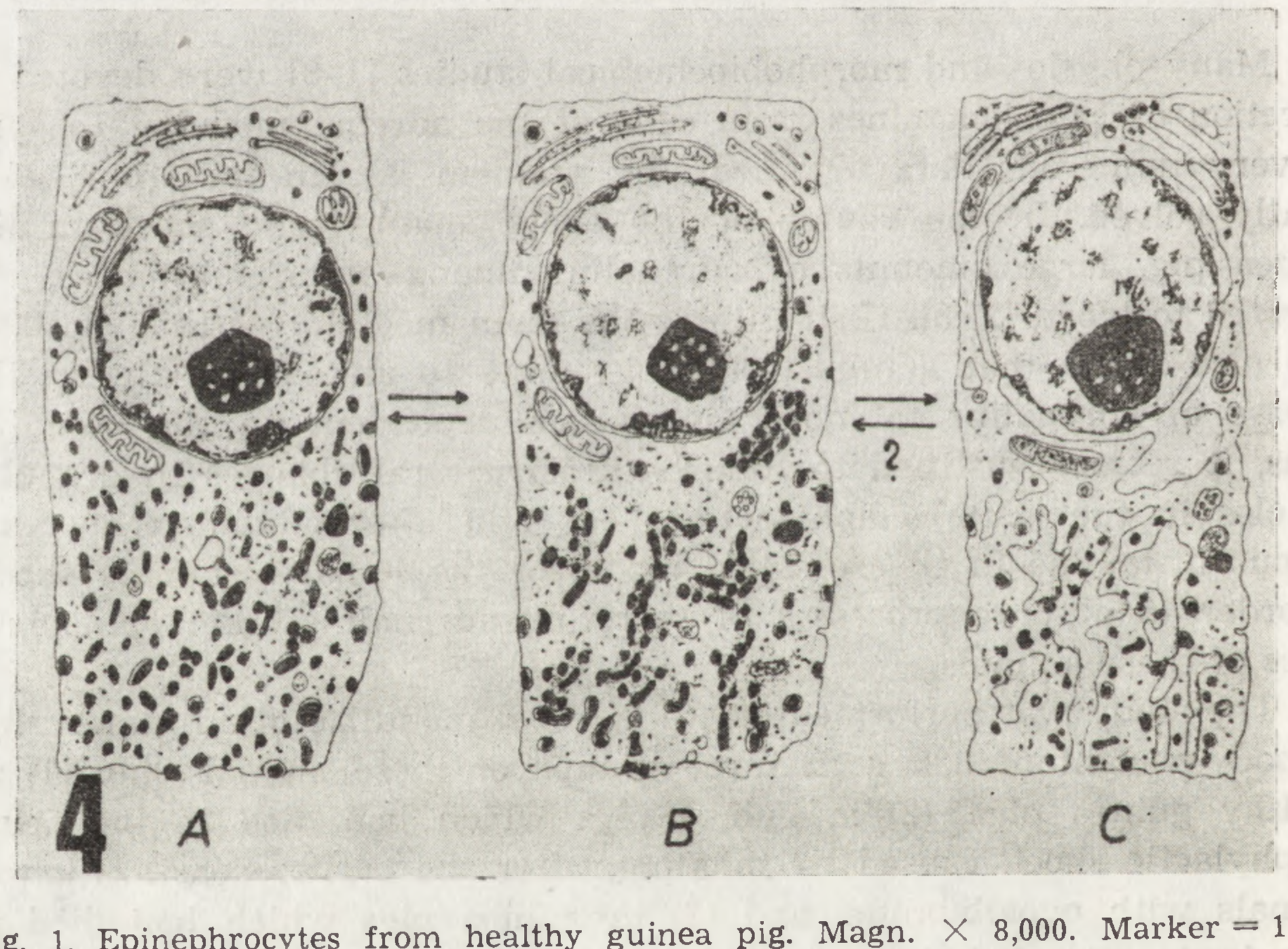


Fig. 1. Epinephrocytes from healthy guinea pig. Magn. × 8,000. Marker = 1 μm
Fig. 2 and 3. Epinephrocytes from guinea pig after acute anaphylactic shock. Details — see text. Magn. × 6,500. Marker = 1 μm
Fig. 4. Model of forming the characteristic system of canaliculi and cisternae from membranes of granular vesicles of epinephrocyte of the guinea pig; *A* — non-stimulated epinephrocyte, *B* — epinephrocyte at the initial stage of functional stimulation, *C* — epinephrocyte after adrenaline secretion in the acute anaphylactie shock

As a result of studies performed in guinea pigs which had died in the acute anahylactic shock, epinephrocytes in submicroscopic pictures showed all modes of adrenaline secretion. There could be observed the transcytoplasmatic diffusion and directly from the granular vesicle through its close connection with the cellular membrane, exocytosis and the expulsion of the whole vesicle. Moreover, characteristic pictures of secretion of this catecholamine were found, which have not been described so far.

Contrary to submicroscopic pictures of epinephrocytes of healthy animals (Fig. 1), the number of almost empty vesicles and vacuoles was much greater. On the other hand, the number of granular vesicles decreased considerably in many epinephrocytes, and the remaining vesicles formed characteristic agglomerations, separated with empty vesicles and vacuoles (Fig. 2). Apart from releasing a certain amount of whole granular vesicles (from very strongly stimulated epinephrocytes), characteristic, irregular (twisted) systems of wide and dilated canaliculi and cisternae, draining large cytoplasm regions, were observed. They were in a very close contact with the "destroyed" segments of the cellular membrane. These structures were sometimes connected with large vacuoles of the Golgi area, with the external nuclear membrane, as well as with membranes of the endoplasmic reticulum (Fig. 3). Similar, but less intensive changes, were observed in the histamine shock. Epinephrocytes of these animals, however, had no canaliculi and cisternae which were so big and connected with one another. Diameters of empty vesicles were large and usually contained more "remnants" of the electron-dense material, than in the anaphylactic shock. These changes were not manifested in the same way in individual epinephrocytes. On the basis of the review of a series of electronograms, the scheme (Fig. 4) was developed, presenting my own model of forming structures of this type, i.e. the formation of the characteristic system of canaliculi and cisternae from membranes of granular vesicles of the epinephrocyte of the guinea pig in the course of the so-called "stimulus-secretion coupling" [1, 5], which is doubtlessly connected with the main adrenaline secretion from these cells in the acute anaphylactic shock. The arising of such structures from a certain amount of granular vesicles, seems to be a slightly different form of exocytosis. It was called the "massive and easier" exocytosis. The secretion occurred in such a way in this case chiefly through formed canaliculi and cisternae. It is just this way that enables very quick secretion of so large amounts of adrenaline in the acute anaphylactic shock [2, 3]. Granular vesicles in this case could be considered to be transitional forms of the reversible system "resting-vesicular/active-tubular" [6]. The obtained results confirm the fact

that histamine is not the only mediator of the anaphylactic shock. Apart from this amine, the stimulating effect on adrenaline secretion by epinephrocytes is shown by bradykinine and SRS-A, and — as it is suggested by some authors — antigen itself [3] can be the additional secretory stimulus.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (437—440)

INFLUENCE OF STARVATION ON THE ACTIVITY UDP-Gal→GlcNAc TRANSFERASE FROM RAT LIVER GOLGI APPARATUS (GA)

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Starvation of rats is often used prior to the investigations of rat liver GA [1, 2, 3, 7]. In some cases, however, the animals are not starved, but the livers are immediately taken for Golgi-rich membrane fraction (GF), isolation or investigation GA in situ.

On the other hand, drastic alterations in the GF yield and in the UDP-Gal→GlcNAc transferase activity and morphology of GA in streptozotocin-induced diabetes was previously found in our works [5, 6]. Dramatic decrease (c. 25%) of body weight in streptozotocin diabetes may correspond to the starvation state. It was therefore interesting to study the influence of starvation on the GF yield, and the activity of the marker enzyme.

Female Wistar rats were fed ad libitum on commercial pelleted food and tap water. Then they were divided into two groups: 1–6 control animals without any treatment, 2–6 animals fasted 16 hrs befor killing. All animals were exsanguinated under light ether anaesthesia, and the livers were immediately washed and used for GF isolation by Fleisher's method [2]. Activity of UDP-Gal→GlcNAc transferase was determined according to Fleischer [3], immediately after isolation of GF. The protein was determined by the method of Lowry et al. [4].

Reagents: Tris and bovine serum albumin were obtained from the Koch-Light Lab., UDP-Gal and Triton X-100 from the Sigma Chem. Co., UDP-U^{14}C-Gal was obtained from the Amersham Radiochemical Centre, Dowex 2X8 (200–400 mesh) came from Fluka. All the remaining reagents were analytical grade products from P.O.Ch.

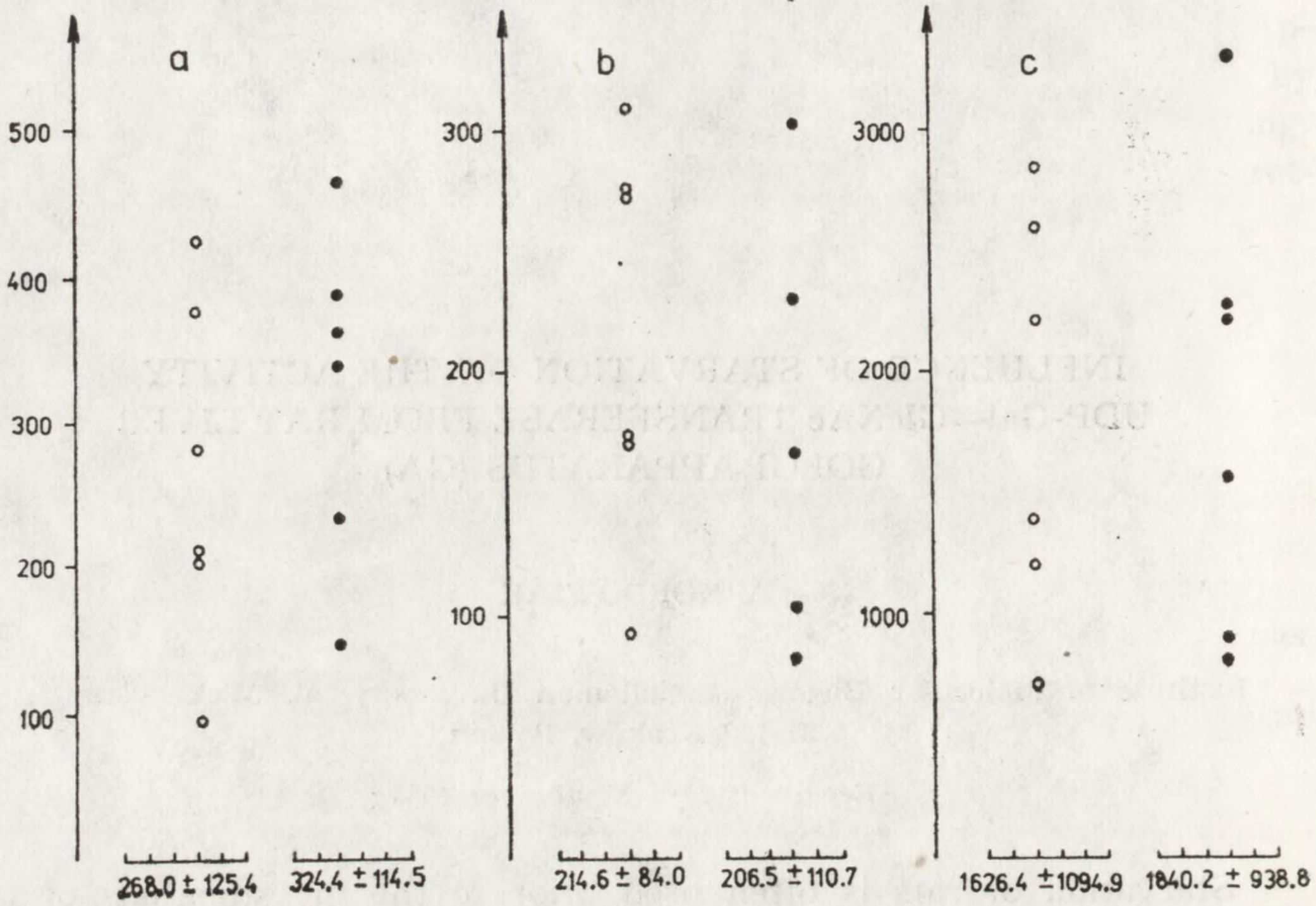

Fig. 1. Activity of UDP-Gal → GlcNAc transferase in the Golgi-rich membrane fraction, originated from the livers of control (○) and starved (●) rats. The activity is expressed in three different ways: *a* — specific activity in nM of galactose transferred per h and per mg of protein, *b* — activity in nM of galactose transferred per h and per g of liver, *c* — total activity in nM of galactose transferred per h and per whole organ. The mean values ± SD and dispersions of individual values are given for each group

In comparison with control (0.83 ± 0.12 mg of protein/g of liver) statistically significant decrease ($0.01 < p < 0.05$) of the GF yield was found in starved rats (0.61 ± 0.16 mg protein GF/g of liver). In starved rats, specific activity of galactosyl transferase was higher than normal, but statistically insignificant. Total activity calculated per whole liver or per g of liver was similar in both investigated groups (Fig. 1).

Starvation influences the yield of GF isolation (calculated as mg of GF protein/g of liver). This is probably due to lower than normal levels of secreted glycoproteins and glycolipoproteins, and/or higher catabolism of these products. Activity of UDP-Gal→GlcNAc transferase the marker enzyme of GF, was similar in control and starved groups of animals.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (441—442)

INHIBITING EFFECTS OF $CoCl_2$, $MnCl_2$ AND $LaCl_3$ ON POTASSIUM — INDUCED REVERSED BEAT OF COMPOUND CILIA IN *STYLONYCHIA MYTILUS*

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Stylonychia mytilus shows reversed beat of compound cilia (Adoral Zone Membranelles and somatic cirri) when exposed to appropriate concentrations of potassium and calcium ions in external medium. It is evident from recent behavioral and electrophysiological studies that the above-mentioned change of the effective beat of compound cilia (i.e. ciliary reversal — CR) is caused by the transmembrane current of free calcium ions from the surrounding medium into cell interior.

Ciliates were transferred 1 hour before starting experimental procedure to solution: 1 mmol/l $CaCl_2$ + 1 mmol/l Tris/HCl (pH 7.3). The preliminary tests revealed that 20 mmol/l KCl prepared on the basis of above-mentioned sol. causes CR of compound cilia lasting 115–165 sec. Experiments were performed with ciliates exposed to 20 mmol/l KCl in presence of various concentrations of $CoCl_2$, $MnCl_2$ and $LaCl_3$ in external medium.

$CoCl_2$ caused statistically significant decrease of CR duration in concentrations 0.2–3.1 mmol/l, while CR response was completely absent at higher $CoCl_2$ concentrations. Similar decrease of CR duration was observed in Stylonychia exposed to 0.4–3.1 mmol/l $MnCl_2$ with absence of CR response in higher concentrations. $LaCl_3$ induced significant decrease of CR duration within wide range of appiled concentrations (0.12–0.16 $\times$ 10^{-3} mmol/l), but CR response was never completely inhibited.

The inhibiting effect of $CoCl_2$, $MnCl_2$ and $LaCl_3$ on potassium-induced CR in *Stylonychia mytilus* suggests that all three tested inorganic agents show more or less expressed blocking effect on hypothetical voltage-sensitive calcium channels, in analogy to similar effects reported in case of other excitable cells in multicellular organisms.

POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (443—444)

ACTION OF CHOLESTEROL ON EXCITABILITY OF *PARAMECIUM OCTAURELIA*

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Paramecium aurelia was the first ciliate shown to have lipid nutritional requirements, namely a sterol [8]. The sterol requirement is marked by a high degree of specifity between molecular structure and biological activity. The same has been established for other members of the genus *Paramecium* [2, 3]. Enrichment of growth medium for *Paramecium octaurelia* with cholesterol caused lowering of plasma membrane permeability for potassium [7], and prolongation of ciliary reversal [CR] induced by increased potassium concentration in the medium [6]. In contrast, *Tetrahymena pyriformis* has no nutritional requirement for sterol and addition of cholesterol to medium led to a reduction in cellular volume [1]. In view of these observations on *Paramecium* a further investigation of motile behavior modification by cholesterol was undertaken in the present report. *Paramecium octaurelia*, strain 299 s, was cultivated according to the modified method of Soldo and Wagtendonk [5] with the use of axenic medium designed by Soldo et al. [4]. Following culture media were applied: 5 μg/ml of stigmasterol (control medium), 3 μg/ml of stigmasterol and 2 μg/ml of cholesterol (CH I), and 1 μg/ml of stigmasterol and 4 μg/ml of cholesterol (CH II). The cells were cultivated in above-mentioned media for six days. Afterwards paramecia were washed and dense ciliate suspension was left in the buffer (1 mM TRIS-HCl and 1 mM $CaCl_2$, pH 7.3) for 12 hours. The duration of the K^+-induced CR was measured with a stopwatch. The obtained results are given in the Table 1. As seen in the Table 1, increase of the medium cholesterol brings about noticeable prolongation of K^+-induced CR duration which was elongated linearly with cholesterol enhancement in the medium. The direct examination of ciliate excitability

TABLE 1

Duration of K^+-induced ciliary reversal response in *Paramecium* in seconds

Medium	32 mM	48 mM	64 mM
control	25.5±1.0	36 ±2.2	43±1.7
medium CH I	29.0±2.6 (11.4%)	39±2.6 (9.3%)	48±1.5 (11.8)
medium CH II	32.0±0.8 (23.1)*	42±1.2 (16.4)	52±2.1 (20.9)

In parentheses are given parcentage deviations of CR time responses from that in control medium.

indicates that cholesterol introduced into cell membrane modifies greatly the behavior of *Paramecium* cells. The presence of this lipid in the higher amount in the cell prolongs the time of backward swimming, i.e. CR, of ciliates under the influence of external chemical stimulus, without producing any changes in other parameters of the cell behavior. On the basis of presented observations it may be concluded that cholesterol effects functional state of the cell membrane of *Paramecium*, presumably through inhibition of hypothetical calcium pump, manifested by prolongation of ciliary reversal response.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (445—446)

INHIBITING ACTION OF SOME BIVALENT AND TRIVALENT CATIONS ON K^+-INDUCED CILIARY REVERSAL IN *FABREA SALINA*

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Paramecia like other ciliate species proved to be excitable unicellular organisms [1] possessing voltage sensitive calcium channels within cell membrane [2]. Recently it was shown that marine ciliate *Fabrea salina* belongs also to group of excitable unicellular organisms, showing behavioral and electrophysiological responses towards external stimuli, based on the occurrence of free Ca ion transmembrane currents [3]. In connection with above-mentioned problems, it appeared interesting to check experimentally how some Ca channel blocking substances affect the response of *Fabrea salina* to external stimuli (KCl) which induce so-called "everlasting ciliary reversal" (ECR), associated with penetration of Ca ions throughout hypothetical calcium channels into cell interior.

Fabrea salina was grown in salt solution medium (1.1 M NaCl + + 0.13 M $MgCl_2$ + 0.025 M $CaCl_2$ + 0.022 M KCl + 0.005 M Tris/HCl — pH 7.2) inoculated with *Areobacter aerogenes*. Before starting experimental procedure, *Fabrea salina* was washed in salt solution without bacterial food.

Fabrea shows ECR when exposed to appropriate concentration of potassium ions in external medium. Under above-described conditions the threshold concentration for inducing ECR was 0.12–0.13 M KCl.

The following inorganic agents ($CoCl_2$, $LaCl_3$, $MnCl_2$, $NiCl_2$) were checked for their possible inhibiting effects on K-induced ECR response in *Fabrea*. All agents were applied at sublethal concentrations for 15 minutes before testing changes of their threshold concentrations for induction of ECR.

$CoCl_2$ and $NiCl_2$ showed marked inhibiting effects on K-induced excitability state of cell, i.e. increase of KCl threshold concentrations for induction of ECR. Effects of $LaCl_3$ and $MnCl_2$ were negligible in this respect. The obtained results support the view that action of Ca channel blockers differs much in dependence on kind of excitable cells.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (447—450)

ULTRASTRUCTURE OF PHOLEM OF POTATO PLANTS INFECTED BY AN ISOLATE OF POTATO LEAF ROLL VIRUS (PLRV)

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Studies of aphid (*Myzus persicae* Sulz.) — borne transmission of potato leaf roll virus (PLRV) have shown that the efficiency of aphid in virus transmission depends on potato plant variety; namely, var. Osa, as compared with var. Pola and Giewont, was an evidently better source of PLRV for aphids [1].

The present studies were designed to gain knowledge of the sequence of cytological changes in phloem cells of potato var. Osa, Pola and Giewont plants infected by a PLRV isolate (L 13B); upon use of this isolate, the differences between potato varieties in their efficiency as virus source for aphids, and in virus detectability by the ELISA test, could well be revealed.

The leaf petiole material was sampled in the 2nd, 4th and 6th week after inoculation, fixed in Karnovsky fixative and embedded in Epon 812. The sections were viewed in a JEM 100C electron microscope.

In the course of the pathologic process, all three investigated potato varieties exhibited the occurrence — in companion cells — of vesicular structures containing a fibrous material (Fig. 1), morphologically resembling nucleic acids; this suggested that the fibrous material may consist of virus RNA, its replicative form or progeny genomes.

Var. Pola showing low efficiency as a PLRV source for aphids was characterized by clear-cut changes in endoplasmic reticulum and on the surface of plasmalemma of companion cells. These changes involved, in the first infection phase, proliferation and increase of ER cisternae (Fig. 2), and then they consisted of an accumulation of osmophilic substance globules on the internal side of ER membranes and on the external side

of plasmalemma (Fig. 3). Virus particles were found only in the necrotic cells of phloem (Fig. 4).

Var. Giewont, also being of low efficiency as a PLRV source for aphids, showed a marked thickening of companion cell walls and a presence of osmophilic substance (mostly in the form of globules) distributed along the plasmalemma and on the internal side of ER membranes (Fig. 5). Within the space between cell wall and plasmalemma, many microtubules occurred. Similarly as for var. Pola, PLRV particles were present only in the necrotic cells of phloem (Fig. 6).

The PLRV — susceptible var. Osa, being most efficient as a source of virus for aphid-borne transmission, displayed accumulation of many virus particles in the functioning phloem companion cells (Fig. 7). The arrangement of PLRV particles was irregular or paracrystalline. The irregular arrangement was rather characteristic of cytoplasm regions surrounded by single membranes, whereas the paracrystalline arrangement occurred also within cytoplasm, in the immediate vicinity of other organellae (Fig. 8). In companion cells containing virus particles, osmophilic globules occurred neither on the external side of the plasmalemma nor within the ER cisternae.

The present results pointed to a relationship between the degree of resistance to PLRV and plant phloem ultrastructure. Moreover, they testified to the possibility of developing cytological criteria for the evaluation of the differences between potato varieties in the reaction to PLRV infection.

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Fig. 1. Vesicular structure with fibrous contents (arrow) in protoplast of companion cell. Var. Pola; magn. × 20 000

Fig. 2. Increased ER cisternae and occurrence of osmophilic substance on the internal side of membrane (arrow). Var. Pola; magn. × 16 000

Fig. 3. Osmophilic substances in ER extensions and on the internal side of plasmalemma (arrow). Var. Osa; magn. × 20 000

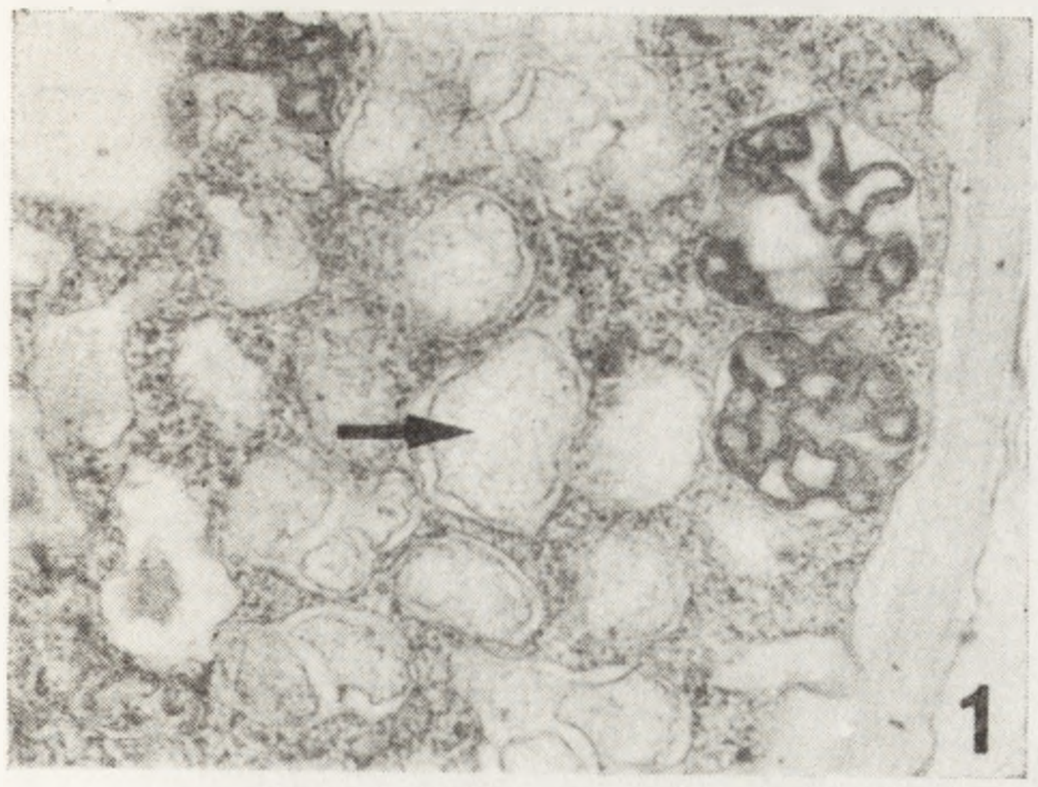
Fig. 4. Virus particles (arrows) in necrotic companion cell. Var. Osa; magn. × 10 000

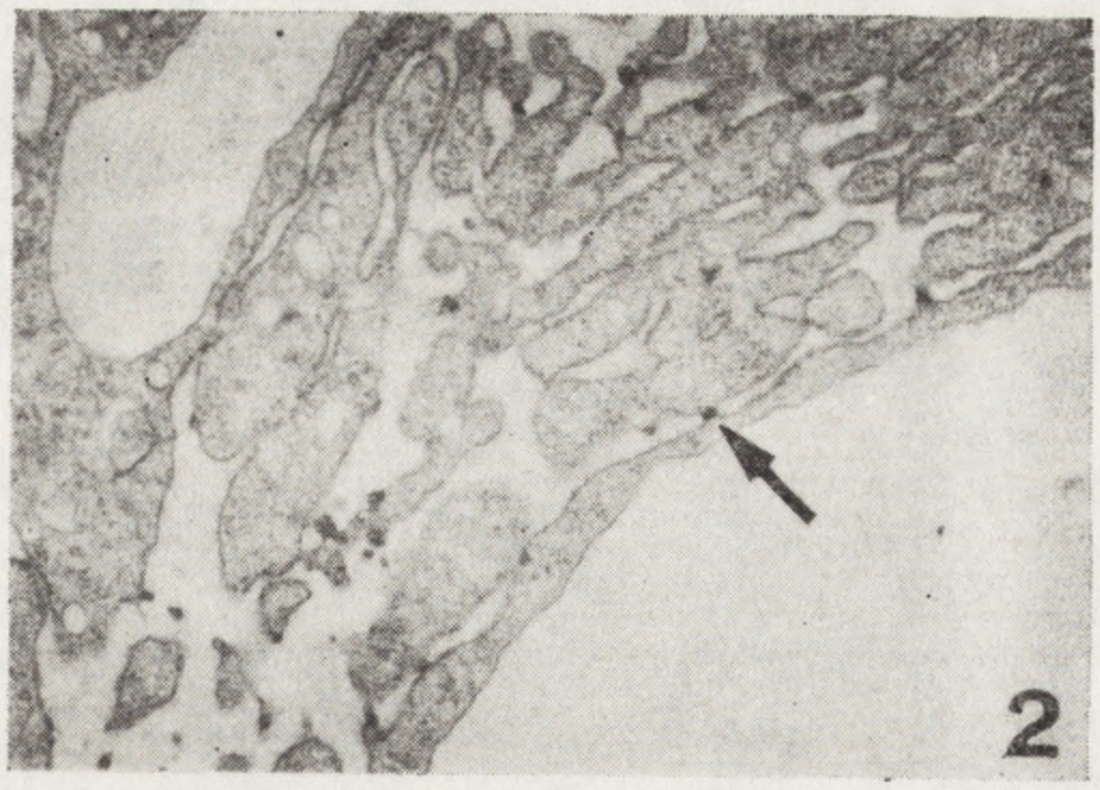
Fig. 5. Extended wall of companion cell (arrow) and osmophilic substance on the external side of plasmalemma of phloem cells. Var. Giewont; magn. × 10 000

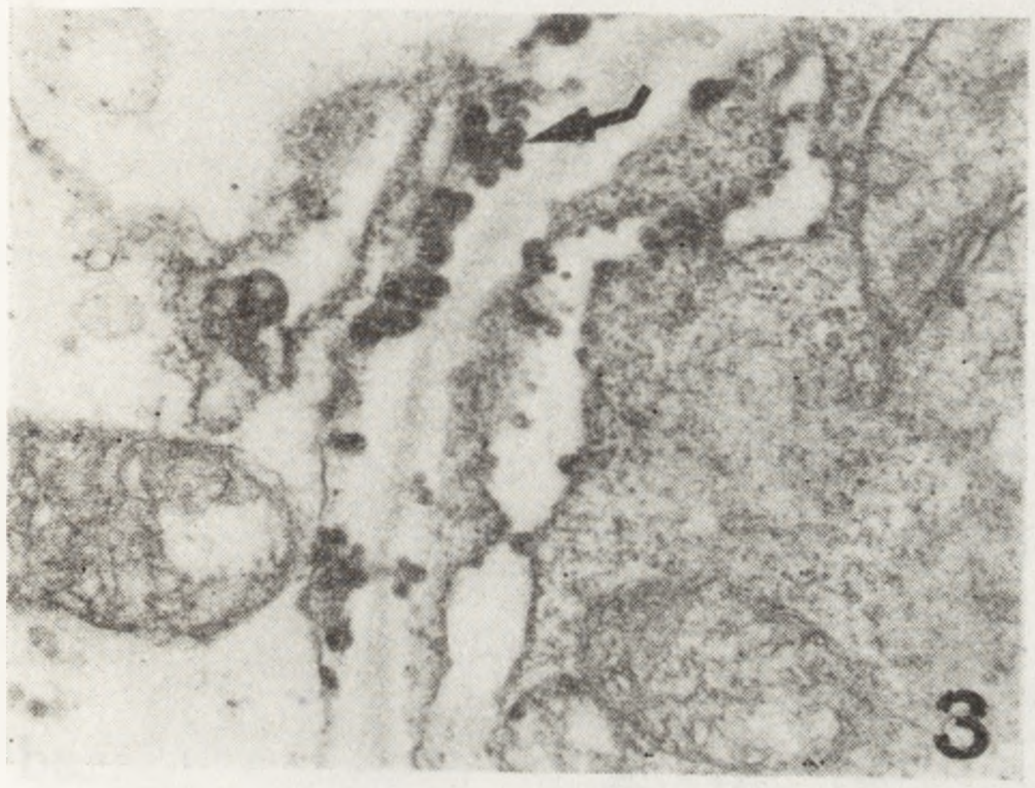
Fig. 6. Virus particles (arrow) in necrotic companion cell. Var. Giewont; magn. × 20 000

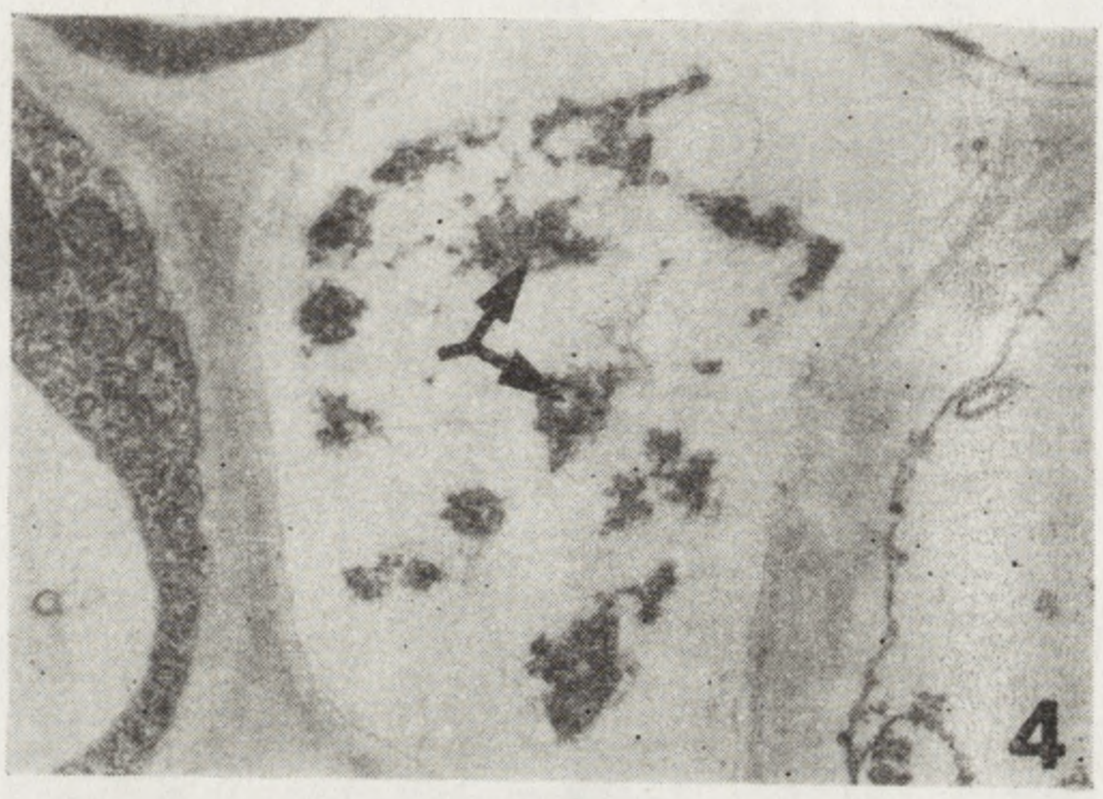
Fig. 7. Fragment of sieve tube, companion cell and phloem parenchyma cell with virus particles (arrow). Var. Osa; magn. × 10 000

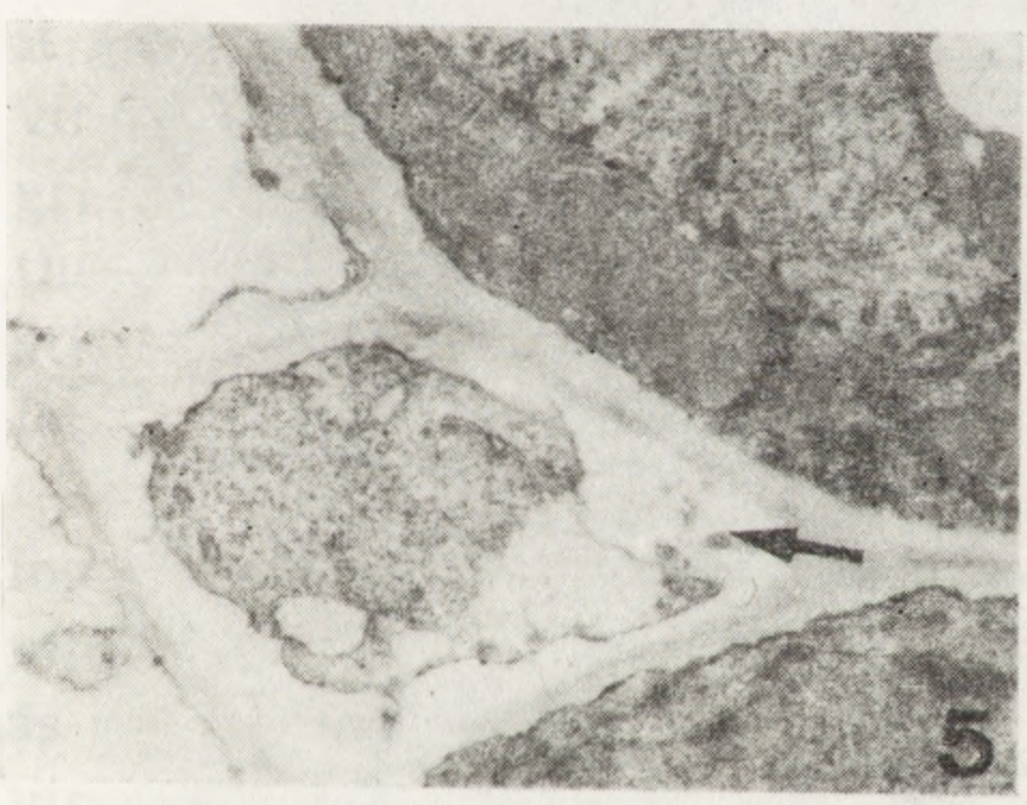
Fig. 8. Paracrystalline arrangement of PLRV particles (arrow) in phloem parenchyma cell. Var. Osa; magn. × 20 000

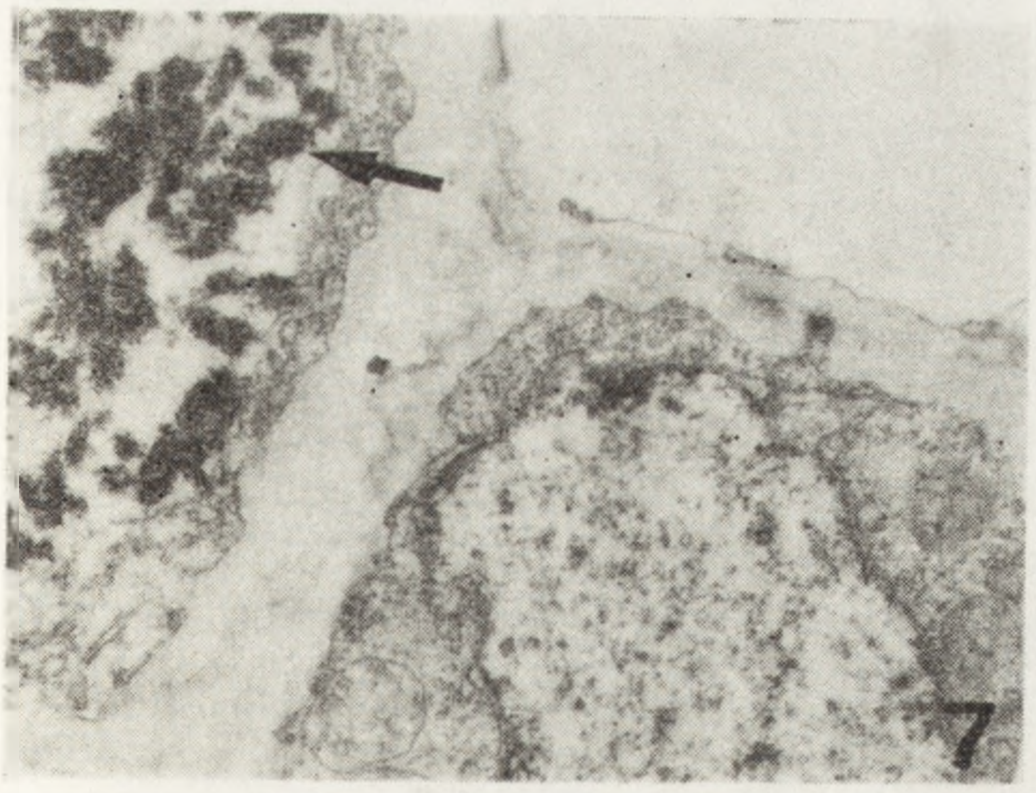


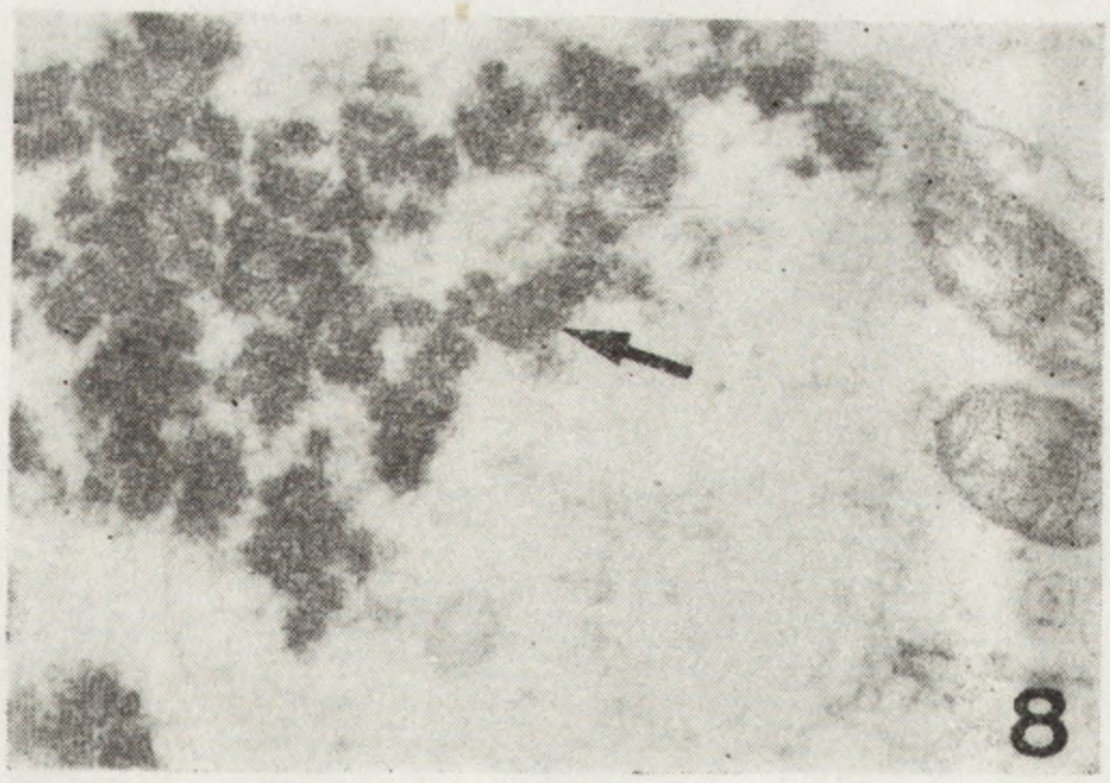












POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (451—454)

REACTION OF POTATO MESOPHYLL CELLS TO THE PRESENCE OF VIRUS PARTICLES INTRODUCED INTO INTERCELLULAR SPACES

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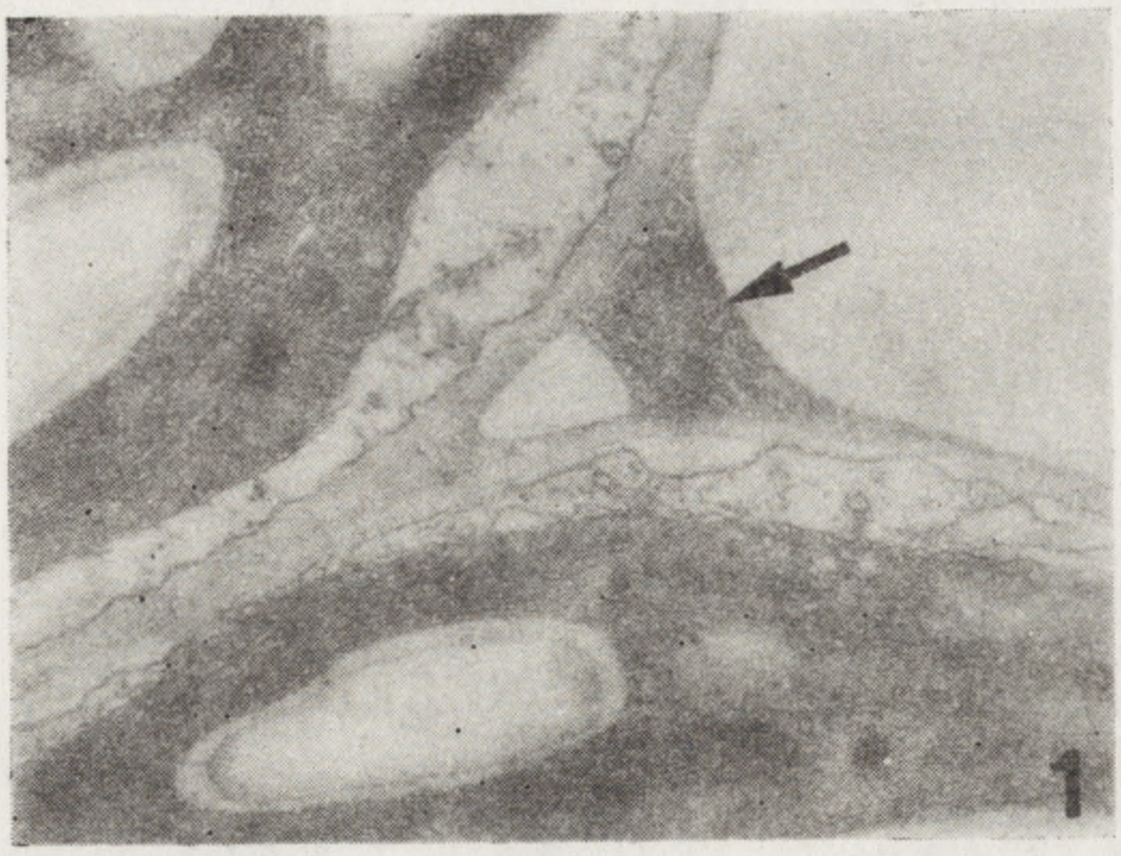
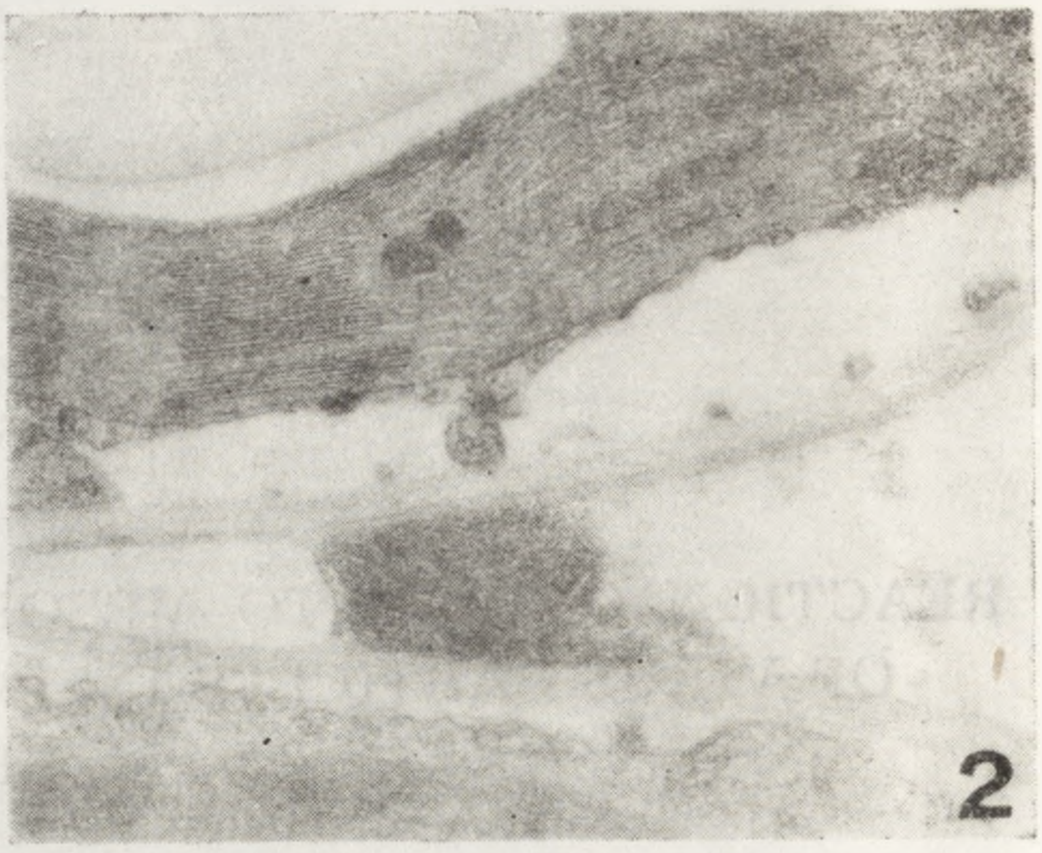
In the virus infection process the cell wall acts as a mechanical barrier protecting the cell from pathogen invasion. On the other hand, it is well known that certain cell wall components, e.g. cuticula, affect virus particles, causing a change in their morphology and gradual degradation. This suggests, on the one hand, participation of cell wall in the plant defense reaction; on the other hand, it may testify to participation of cell wall in the preliminary phase of infection, i.e. in separation of protein capsid from virus genome.

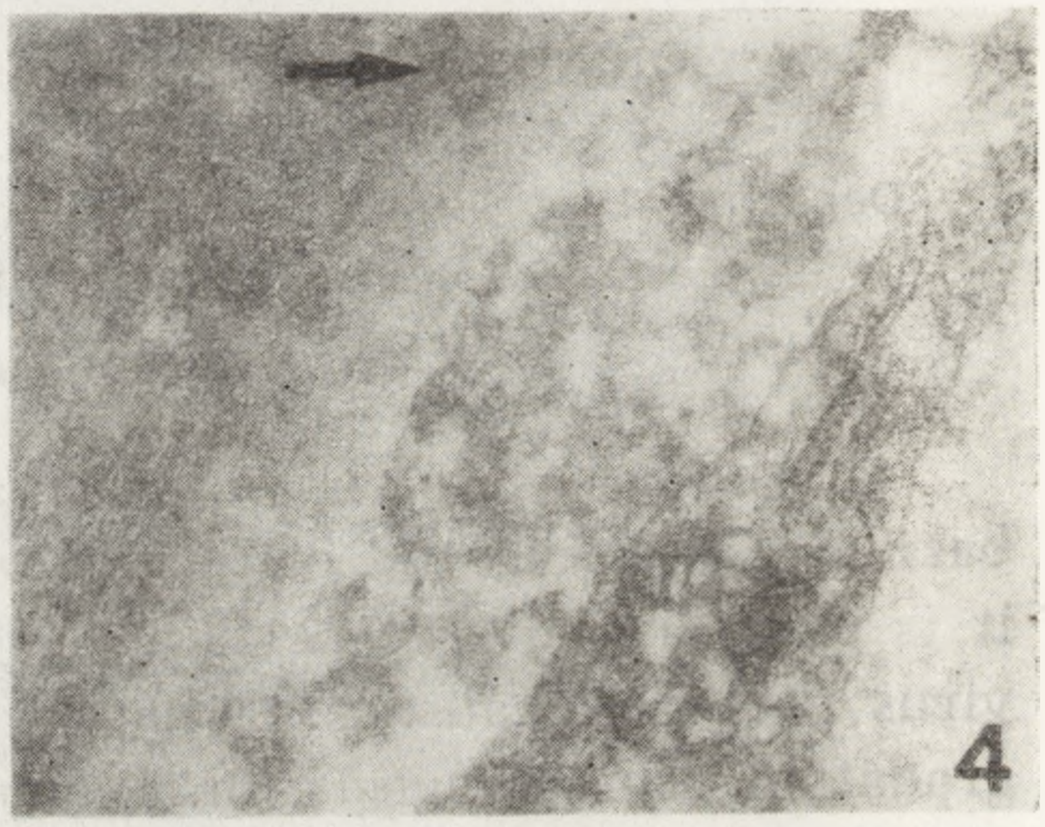
The present studies were aimed at observing changes in the morphology of virus particles being in contact with cell wall, as well as determining whether virus particle degradation is influenced by factors such as genetically determined resistance, or susceptibility of plants to a given virus.

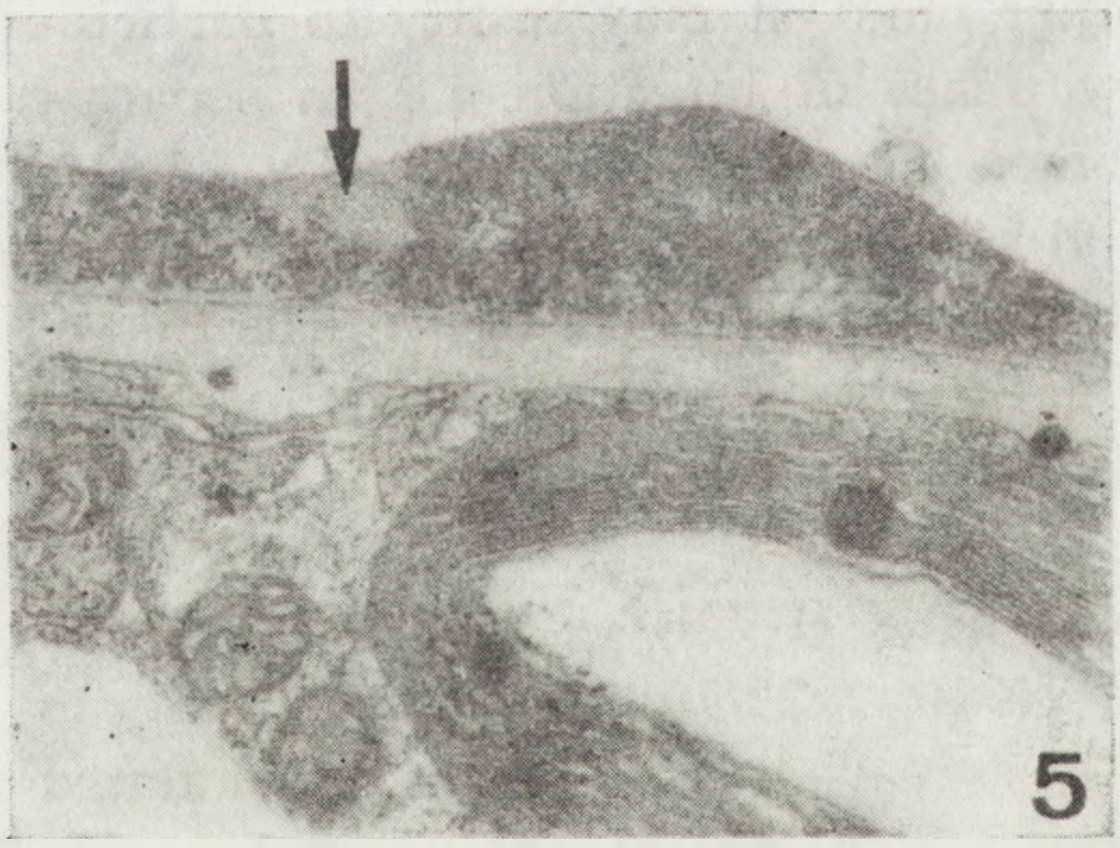
The experiment involved potato virus X (PVX) — inoculated plants of 2 potato varieties: resistant to PVX (cv. 22/70), and susceptible to this virus (var. Flisak). Plants of these varieties were also inoculated with tobacco rattle virus (TRV), for which potato plants fail to be a specific host.

A suspension of PVX particles in 0.01 M citrate buffer was introduced with a microsyringe into intercellular spaces of leaf blades. Subsequently, from these leaf blade fragments samples were taken after: 0 h, 1 h, 6 h, 1, 2, 3, 5, 10 and 15 days. The material was fixed in Karnovsky fixative, dehydrated in ethanol and propylene oxide, and embedded in Epon 812. Observations were taken in a JEM 100C electron microscope.









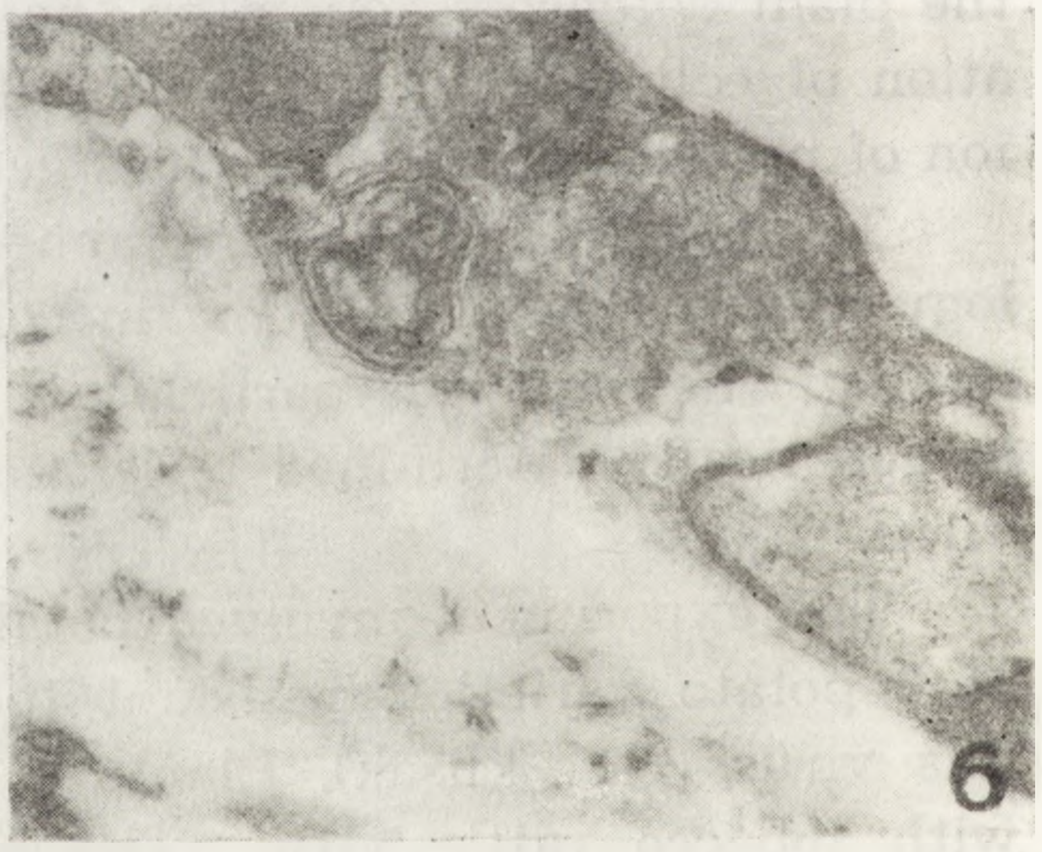


Fig. 1. Arrows show PVX particles with typical morphology. Var. Flisak, 3 days after inoculation; magn. × 20 000

Fig. 2. Aggregate of partly degraded PVX particles. Var. Flisak, 10 days after inoculation; magn. × 20 000

Fig. 3. Aggregates of partly degraded PVX particles. Arrow points to fibrous material. One of the cells exhibits the presence of vesicular structures located near PVX (asterisc). Cv. 22/70, 3 days after inoculation; magn. × 20 000

Immediately after introduction of the suspension containing PVX or TRV into intercellular spaces, virus particles were found to be dispersed in these spaces. At the later times of sampling, virus particles gradually accumulated along cell walls.

PVX particles introduced into intercellular spaces of plants of the susceptible variety (Flisak), for a long time retained their characteristic shape and size (Fig. 1). Only 10 days after inoculation, apart from virus particles, a fibrous material testifying to PVX degradation occurred (Fig. 2). At the same time, essential changes were found neither in cell wall, being in contact with virus particles, nor in cell protoplast. The process of PVX particle degradation began much earlier in the plants of the resistant variety (cv. 22/70). As early as on the 3rd day after inoculation, close to the virus particles a fibrous material being a remainder of the degraded particles, appeared (Fig. 3). Simultaneously, in the vicinity of the cell walls, in the cytoplasm granular ER cisternae were present, and apart from plasmalemma, many small vesicles and microtubules occurred (Fig. 4).

TRV particles, being unspecific for potato plants, after introduction into intercellular spaces, were rapidly degraded in plants of both varieties. The first degradation symptoms were noted as early as on the 3rd day after inoculation (Fig. 5). In cell protoplasts, numerous microtubules and granular ER cisternae were visible; outside of plasmalemma, myelin structures were observed (Fig. 6).

The present results lead to the conclusion that virus particles introduced into intercellular spaces become degraded in contact with cell wall. This process is accelerated in plants not being a specific host to the given virus, or in plants with genetically determined resistance to this virus. In these plants, virus particle degradation is associated with changes in the morphology of cell protoplasts.

Fig. 4. Arrow indicates fibrous material derived from degraded PVX particles. In cytoplasm granular ER cisternae are visible. Cv. 22/70, 10 days after inoculation; magn. × 20 000

Fig. 5. TRV particle aggregate on surface of cell wall. Arrows show fibrous material derived from degraded TRV particles. Var. Flisak, 3 days after inoculation; magn. × 20 000

Fig. 6. Fragment of mesophyll cell. Between plasmalemma and cell wall, myelin structure is visible. Cv. 22/70, 3 days after inoculation; magn. × 20 000

POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (455—458)

HCN FORMATION DURING PHAGOCYTOSIS BY HUMAN POLYMORPHONUCLEAR GRANULOCYTES

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Human polymorphonuclear granulocytes (PMN) produce HCN during phagocytosis [1]. Since resting PMNs do not exert this activity therefore HCN should originate in postphagocytic events. It was suggested that production of HCN is a consequence of the chlorination process, namely chlorination of glycine and/or N-glycylpeptides [2, 3].

The chlorination process in phagocytosing PMN has been demonstrated [4, 5]. It occurs owing to myeloperoxidase-mediated HOCl formation from Cl^-, oxidized by H_2O_2. It has been found that the amount of HCN evolved during phagocytosis depends on the strain of bacteria used. Engulfing of *Staphylococcus* epidermidis results in about a doubling of the level of HCN production, compared with that produced in the presence of *E. coli* [1]. Since *Staphylococcus epidermidis* contains glycylseryl peptides in peptidoglycan of cell wall it was reasonable to expect that at least part of the HCN evolved originates from chlorination of non-crosslinked N-glycyl peptides of bacterial cell wall. Indeed, it was found that chlorination of *Staphylococcus epidermidis* by isolated

TABLE 1

No	NaOCl added	N-chloro groups as Cl^{+1}	HCN evolved	HCN / Cl incorporated
	nmoles/6.6×10^8 cells			%
1	302	13.3	2.05	15.4
	518	34.2	3.45	10.0
	777	54.0	3.67	6.8
2	302	21.0	4.4	21.0
	518	50.0	7.2	14.3
	777	74.0	9.2	12.4

MPO-H_2O_2-Cl^- system or by HOCl resulted in formation of chloramines of bacterial cell wall components, followed by HCN release (Table 1, Fig. 1).

Derivation of HCN from the N-terminal dichloroglycyl residues was additionally proved by chlorination of *Staphylococcus epidermidis* grown in the medium with sublethal doses of penicillin (20 mg/ml). It is known that penicillin inhibits transpeptidase crosslinking pentaglycyl peptides, therefore in the presence of penicillin the amount of N-terminal glycyl residues increases. *Staphylococcus epidermidis* cells damaged by penicillin when chlorinated, release 2–3 times more HCN than in the case of uninjured bacteria (in Table 1, no 2, Fig. 1). At last, *S. epidermis* grown in medium containing [2-^{14}C] glycine, release H^{14}CN after chlorination by HOCl or MPO-H_2O_2-Cl^- system. In latter experiments, it was possible to calculate dilution of hot glycine incorporated into bacteria "body".

Application of damaged by penicillin and [2-^{14}C] glycine-containing *S. epidermis* for challenging PMN, proves that part of the HCN liberated during phagocytosis originates from glycyl residues of bacterio components, and that it occurs due to chlorination. It was found that during

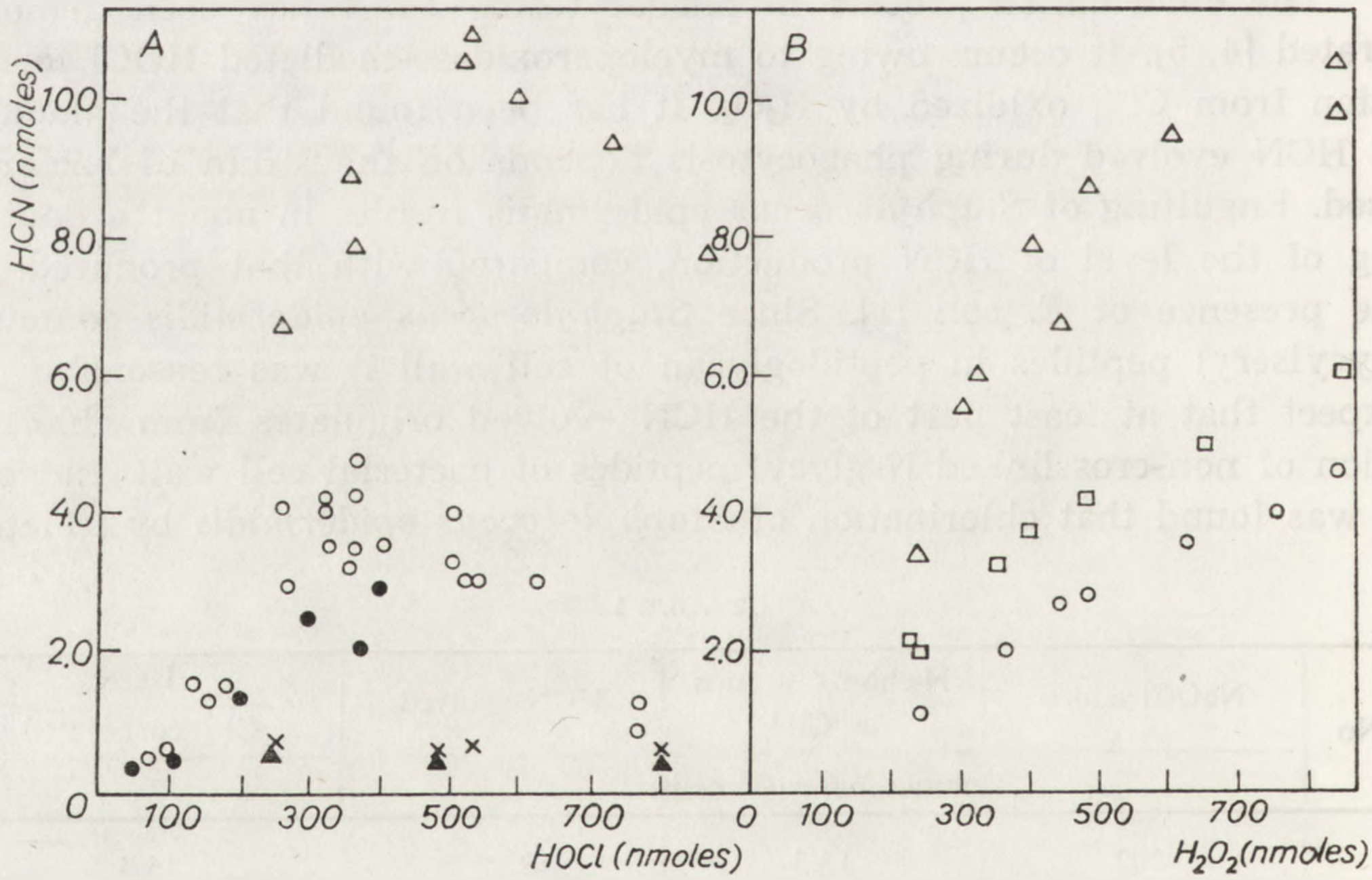

Fig. 1. o — Chlorination of *S. epidermis* (6.6 × 10^8 cells) in 0.1 M acetate buffer, pH 5.3, and 0.1 NaCl (*A*) by HOCl, o at pH 4.05 ●; (*B*) by MPO-H_2O_2-Cl^- system, o MPO — 0.15 μM, □ — MPO 0.48 μM; △ — Chlorination of St. epiderm. (6.6 × × 10^8 cells) damaged by penicillin, in B/MPO — 0.15 μM; Chlorination of *E. coli* (4.2 × 10^8 cells), normal × and grown in the presence of penicillin ▲

phagocytosis of penicillin-treated bacteria, significantly more HCN is produced than during phagocytosis of undamaged bacteria (Fig. 2). The [2-^{14}C] glycine-containing bacteria when phagocytized deliver

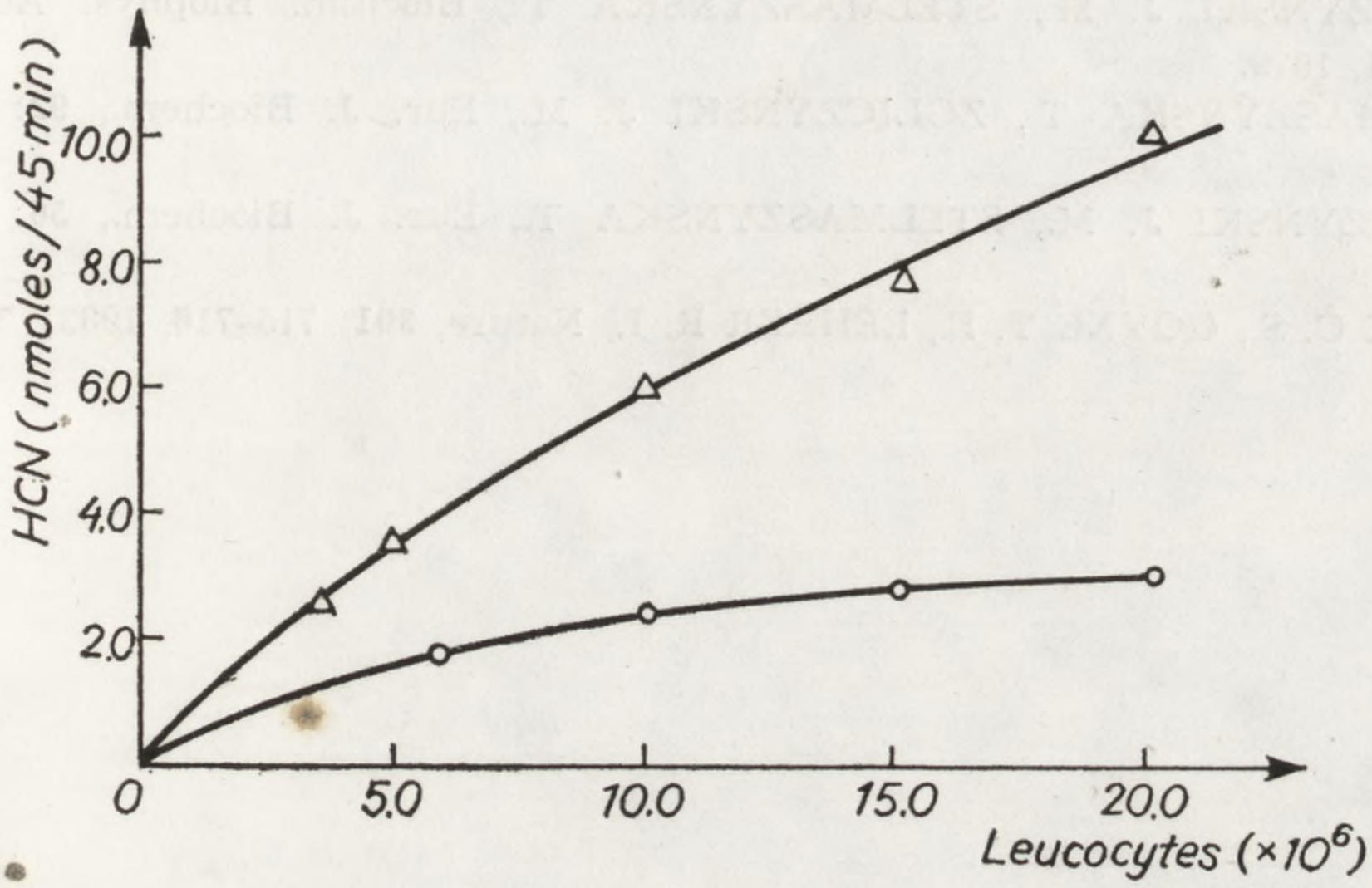

Fig. 2. HCN formation during phagocytosis of *S. epidermis* normal O and damaged by penicillin △. Leucocytes from one donor incubated with bacteria (1.3×10^8 cells) for 45 min. at 36°C

H^{14}CN, and the quantity of HCN is consistent with previously assessed extent of chlorination [4]. The chemically estimated HCN evolved during phagocytosis is higs, if compared with radioisotopical evaluation. Therefore, there should be another source of HCN during phagocytosis. The most likely one seems to be the oxidation of thiocyanate present in plasma. It was found that chloramines oxidize SCN^- according to eq.:

$$SCN^- + 3\ RNHCl + 4\ H_2O \rightarrow HCN + HSO_4^- + 3\ RNH_3^+ + 3\ Cl^-.$$

This is true for chloramines of taurine, N-glycyl peptides and chlorinated bacteria. Production of HCN from thiocyanate makes a metabolic cycle, since (as is commonly known) HCN is converted to SCN^-. Production of HCN from glycyl residues would be anaplerotic to this cycle. It is worth noting that release of HCN by phagocytosing PMN may enhance their bactericidal activity, but does not affect PMN, since these cells are HCN-resistant.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (459—462)

THE INFLUENCE OF PLASMA FACTORS ON CHEMILUMINESCENCE (CL) OF POLYMORPHONUCLEAR GRANULOCYTES (PMN)

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Since the first observation made by Allen [1], CL of phagocytosing PMN has been investigated in many aspects [2]. Now it has been established that efficiency of CL reflects bactericidal ability of PMN [2]. Till now, however, mainly CL of isolated PMN has been measured. Recently, a new method which allows to trace CL in whole blood has been introduced [3]. It makes possible to avoid artificial results coming from isolation procedures and to measure activity of PMN in their natural environment. Using this new methodical approach it was found that PMN in whole blood have low phagocytic and CL activity, i.e. after challenging by polystyrene granules PMN develop a low and delayed CL response. Autoincubation of whole blood however, results in substantial increase in activities of PMN. PMN after incubation in whole blood were prepared (initially activated) to develop considerably higher and accelerated CL response after challenging by granules. After 1.5–2.5 hours of blood incubation at room temperature CL of PMN becomes 5–20 times higher than that measured immediately after venipuncture. This observation suggests that "fresh" blood contains an inhibitor of PMN activity.

The inhibitor is a component of plasma, and is inactivated in the presence of blood cells since autoincubation of plasma does not change its inhibitory power. Most probably, the inhibitor originates from tissue other than blood, because its action is most pronounced in "fresh" blood. The CL activity increases immediately after washing out blood cells from plasma. The activation resulting from washing the blood cells ne-

ver reaches the level of activation observed after autoincubation. Moreover, the duration of CL after washing decreases significantly. The observation indicates that plasma contains not only the inhibitor but also activators of CL.

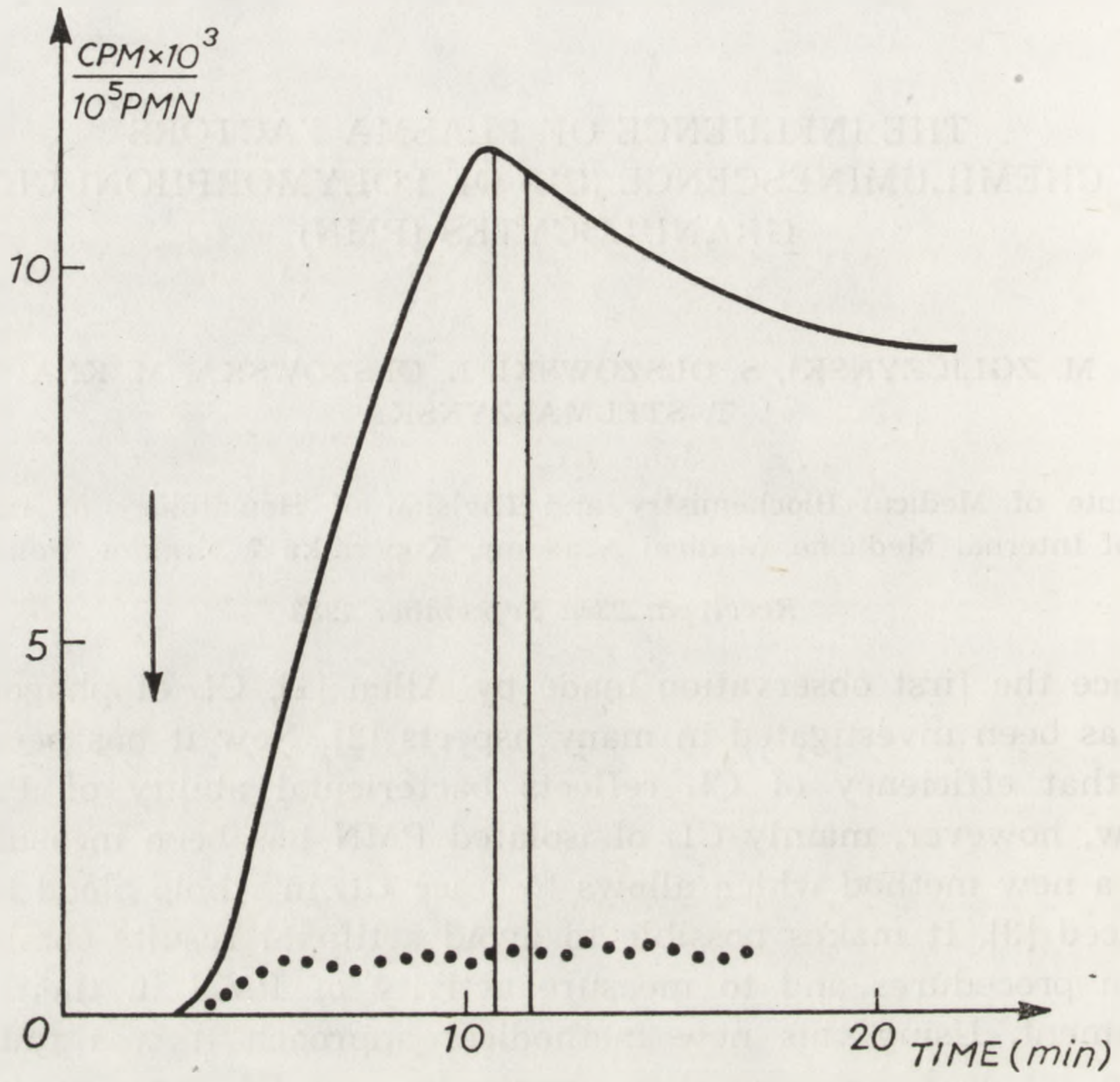

Fig. 1. The reaction mixture contained 0.8 ml of conc. Krebs-Ringer solution, pH 7.4, 0.1 ml 0.28 M glucose, 2.5 ml 1.4 mM luminol, 1.5 ml H_2O and 0.2 ml polystyrene granules added at time indicated by arrow. The CL response measured in a scintillation counter in the IN-coincidence. Solid line — 20 µl of blood, dotted line — 20 µl of blood + 20 µl of plasma

The electrophoretic separation of plasma allows us to find a substance with inhibitory activity on CL within α_1 globulin fraction. On the other hand, however, antitrypsine present in this fraction elicits activatory effect on CL. The preliminary results indicate that PMN are suppressed in circulating blood, and probably can be initially activated when leaving the bloodstream for the inflammatory focus before the final activation by contact with bacteria. The individual variations observed in the inhibitor and activator level, and also in autoactivation ability are promising for more precise evaluation of individual resistance to infection.

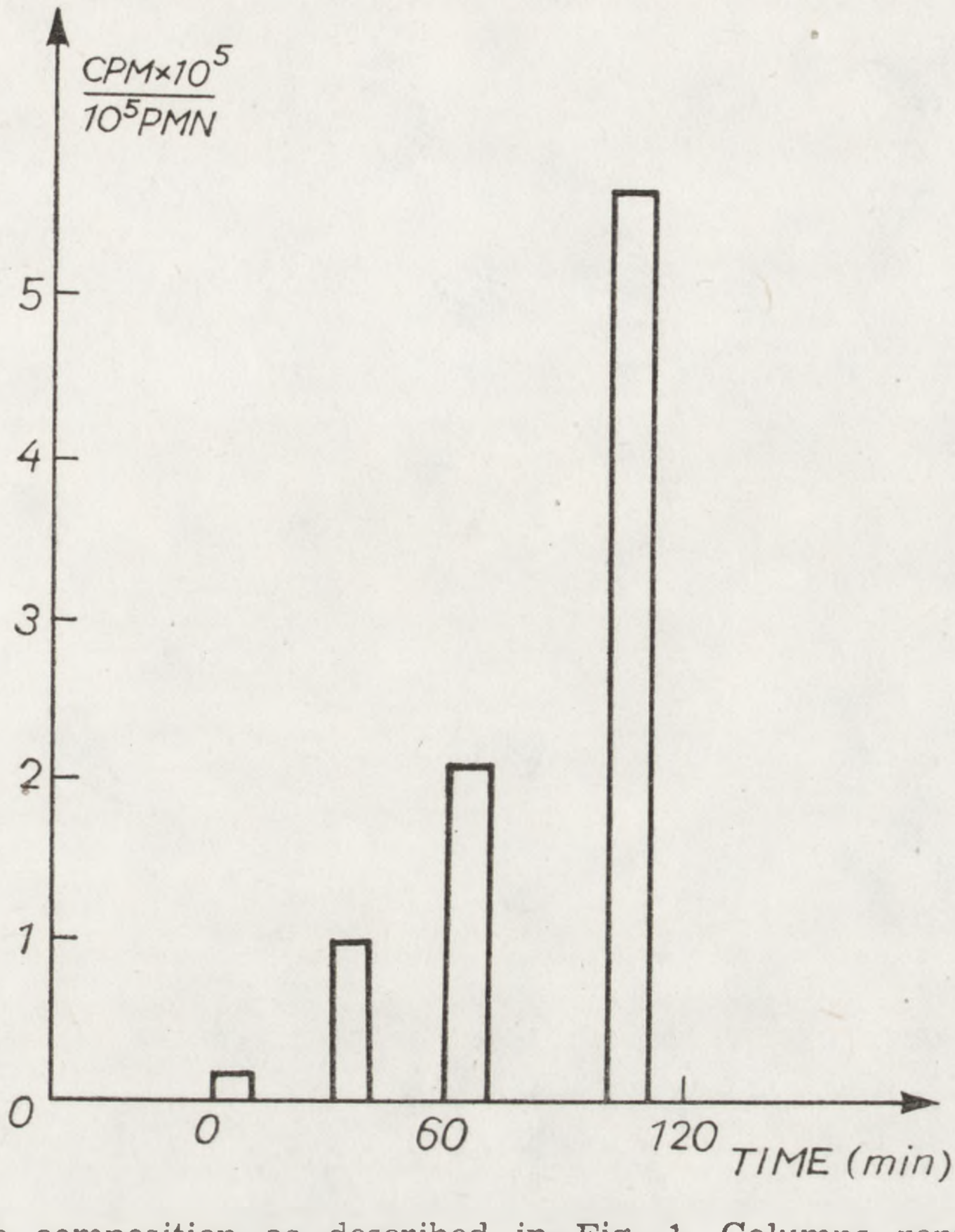

Fig. 2. Sample composition as described in Fig. 1. Columns represent maximal CL response of blood versus time of autoincubation after venipuncture

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (463—466)

II. NEUROHYPOPHYSEAL GLIAL CELLS IN VITRO FOLLOWING INFUNDIBULUM TRANSECTION

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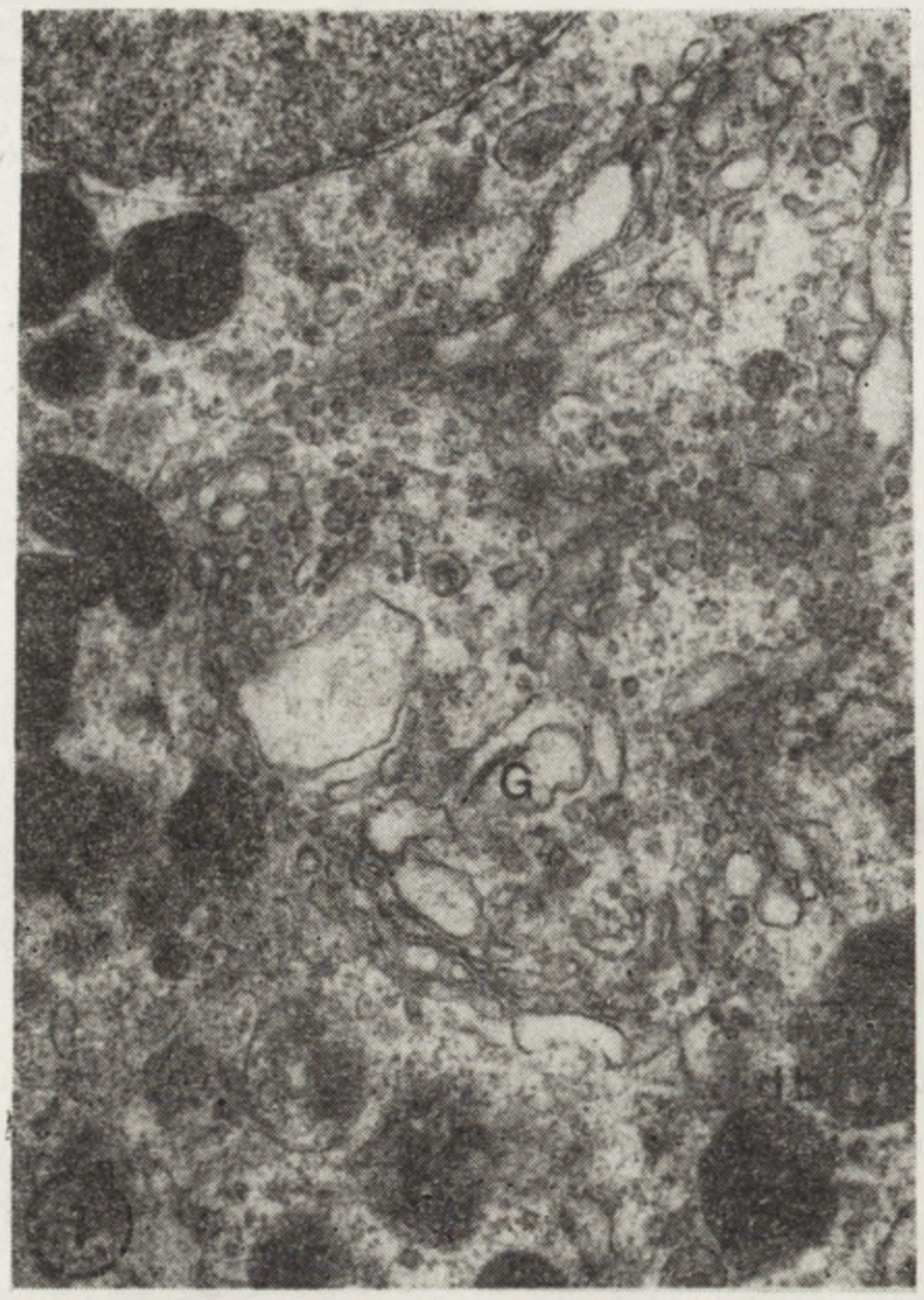
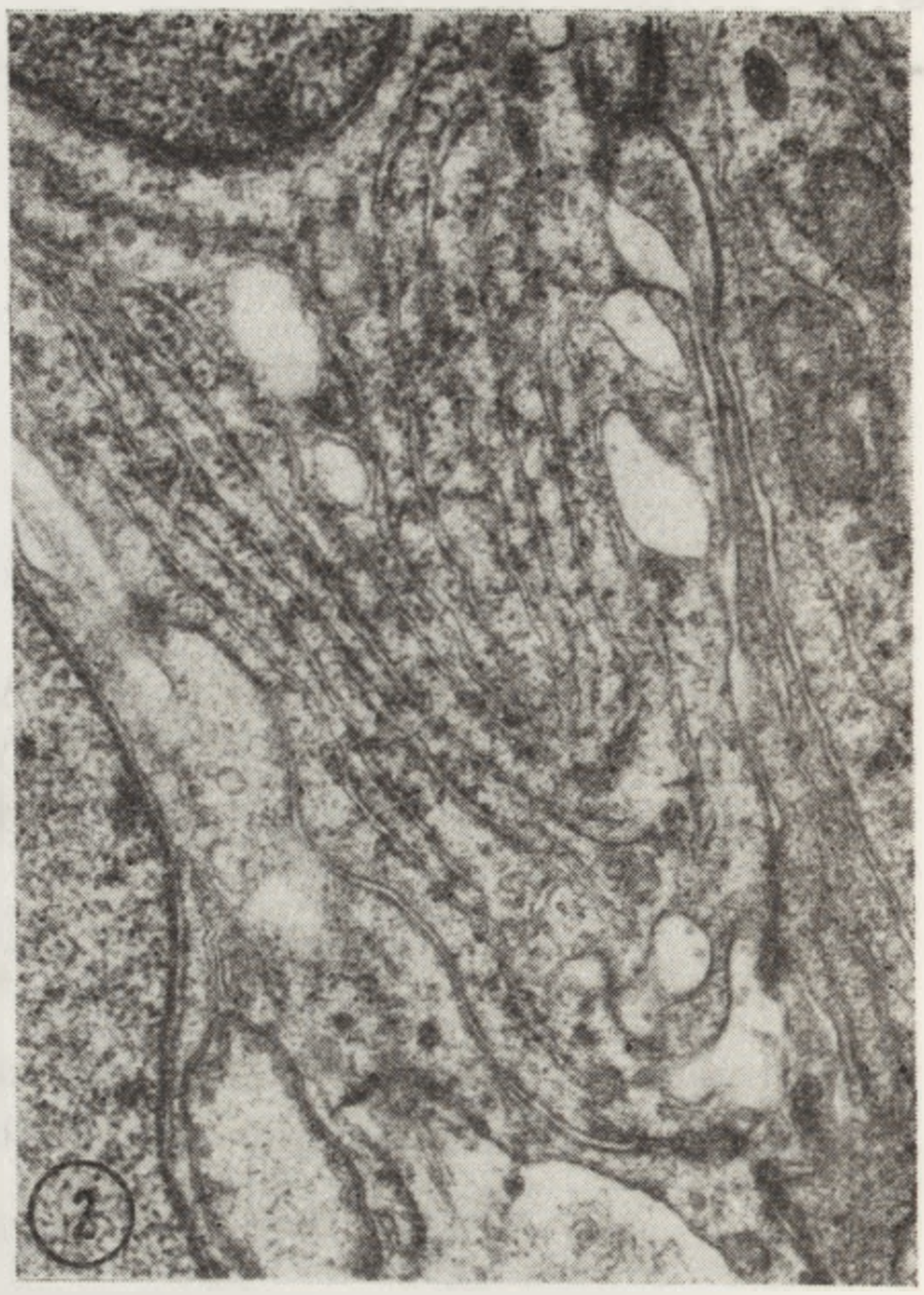
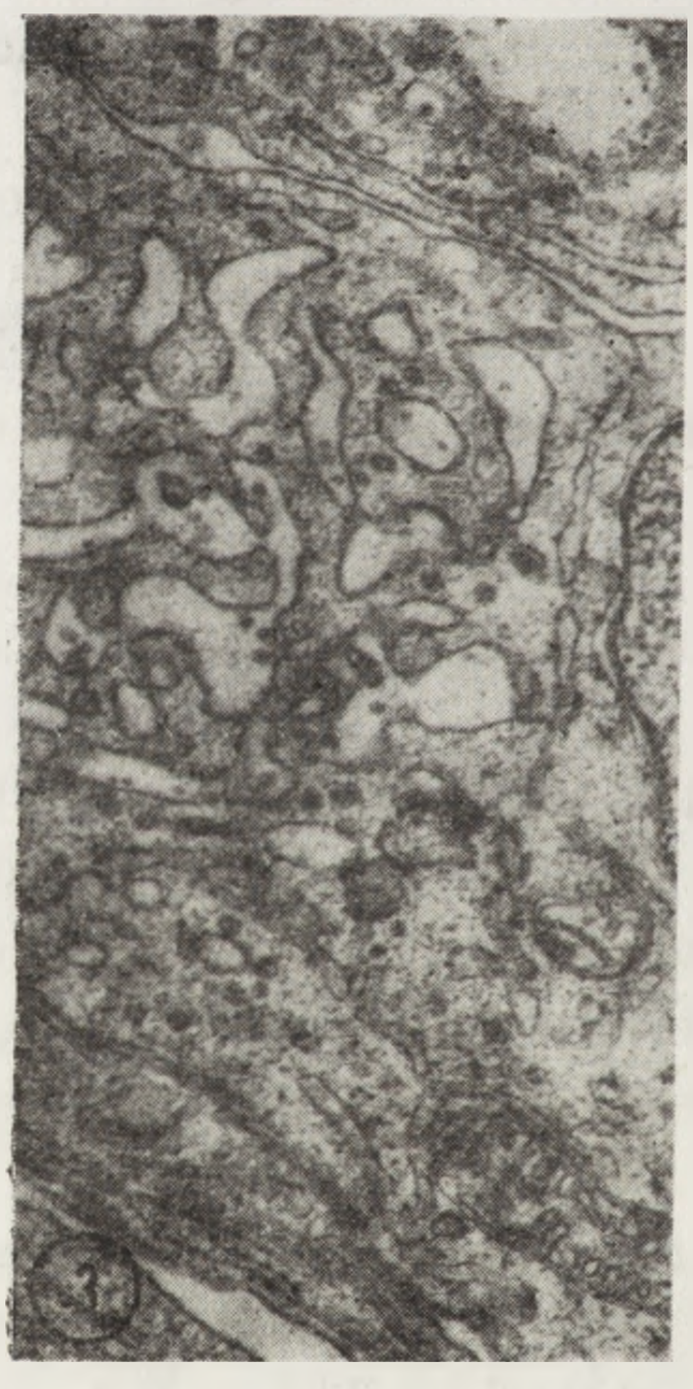
The occurrence of a functional relationship and mutual interaction between hypothalamic secretory neurons and pituicytes, specialized glial cells of the neural lobe, is generally accepted. An organotypic neural lobe culture is a convenient model for investigations of pituicytes devoid of neurosecretory axons. In the present study ultrastructural changes in pituicytes in organotypic neural lobe cultures aged 1–30 days postnatum are reported.

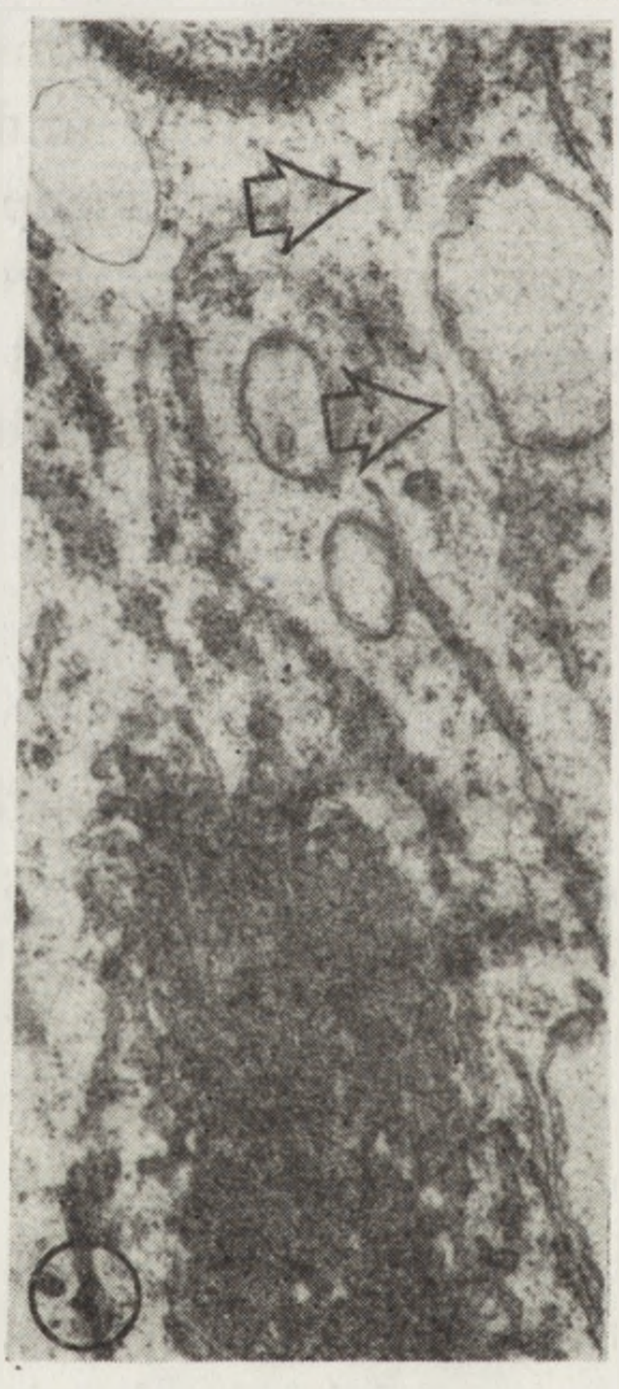
The organotypic cultures were prepared from newborn Wistar rats neural lobes and cultured as previously described. They were examined electron microscopically after 1, 3, 7, 14, 16, 21 and 30 days in vitro (DIV).

Our study revealed phagocytotic properties of immature pituicytes. Between 1–14 DIV, i.e., during phagocytosis of degenerating neurosecretory axons, the pituicytes appeared to be functionally very active cells (Fig. 1). Hypertrophy of the Golgi complexes accompanied by a great number of lysosomes, dense bodies and vesicles may have reflected an increased synthesis of lysosomal enzymes and particularly active cellular digestion. After 14 DIV, the pituicytes contained a moderate number of mitochondria, numerous ribosomes, polyribosomes, prominent Golgi complexes, numerous vesicles and microtubules. The rough endoplasmic reticulum (RER), well developed as a rule, was represented by numerous large and ribosome-studded cisternae lying parallel to one another and forming arrays in the cytoplasm (Fig. 2). Some of the RER profiles were dilated and contained a finely granular electron-dense material. Between 14–30 DIV, there was a progressive increase in the proportion of cells which showed dilatation of the RER and an enlargement of the Golgi complex (Fig. 3). Also observed was an impressive









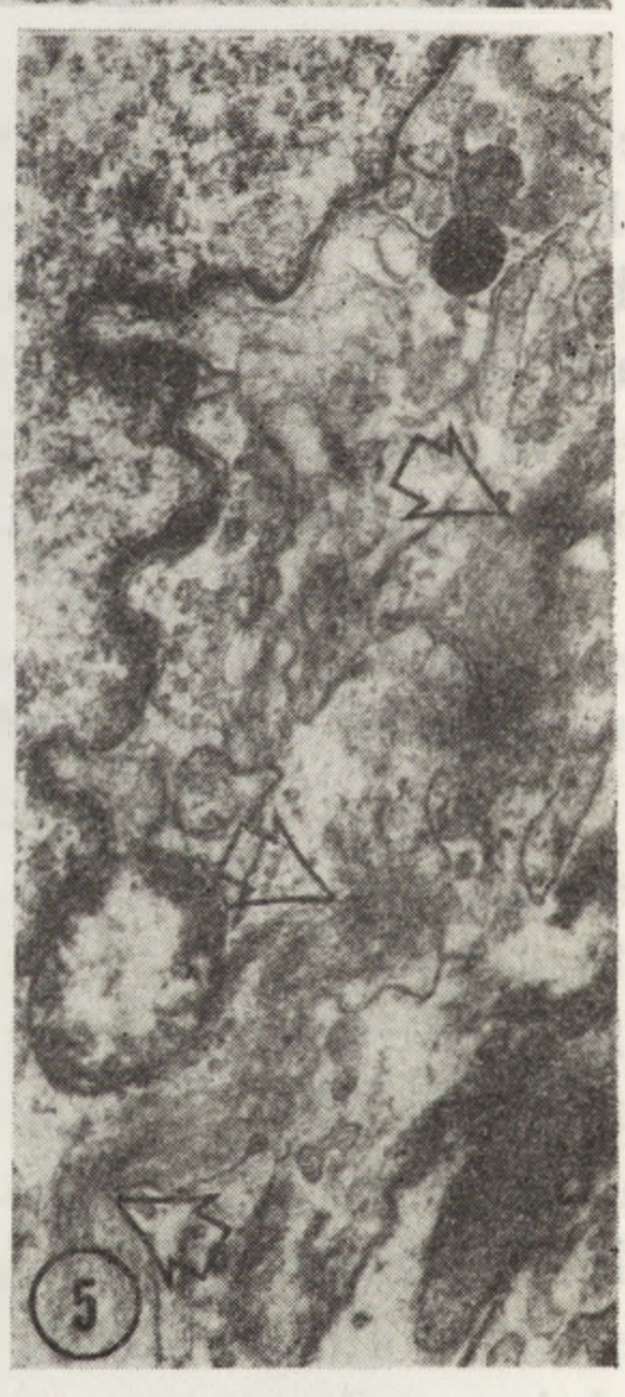


Fig. 1. Reactive pituicyte of 3 DIV culture. Note large Golgi complex (G) with many vesicles and dense bodies (db). × 12 000

Fig. 2. Pituicytes of 14 DIV culture. Note well developed RER and gap- and desmosome—type junctions interposed between the cells. × 13 350

Fig. 3. Pituicyte of 16 DIV culture. Dilated Golgi cisternae with electron-dense vesicles in their vicinity are visible. × 13 350

Fig. 4. Pituicytes of 16 DIV culture. Note SER tubules filled with electron-dense material and microtubules (arrow). × 13 350

Fig. 5. Pituicyte of 30 DIV culture exhibiting distinct tangles of filaments in cytoplasm (arrows). × 13 350

amount of smooth endoplasmic reticulum (SER) in contrast to its relative scarcity in younger cultures. The SER formed a three-dimensional network of tubular elements. In some cells it appeared as a system of long, thin, parallel running tubules filled with an electron-dense substance (Fig. 4). At the same time there was an increase in the number of granulated vesicles, some of which seemed to be Golgi- or SER system- derived. This observation could suggest that the SER may have a function similar to that of the Golgi complex, i.e., rapid transport, concentration and packaging of various substances. In the somata of 21 DIV pituicytes an apparent mesh of filaments appeared, while the majority of 30 DIV pituicytes displayed many tangles of filaments in the cytoplasm (Fig. 5). It seeems probable that directly after axon phagocytosis the enhanced function of pituicytes may be related to the production of some protein substances needed for rebuilding pituicyte structures or proteins for filament formation.

POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (467—470)

I. MORPHOLOGY OF RAT NEUROHYPOPHYSEAL GLIAL CELLS MATURING IN VITRO

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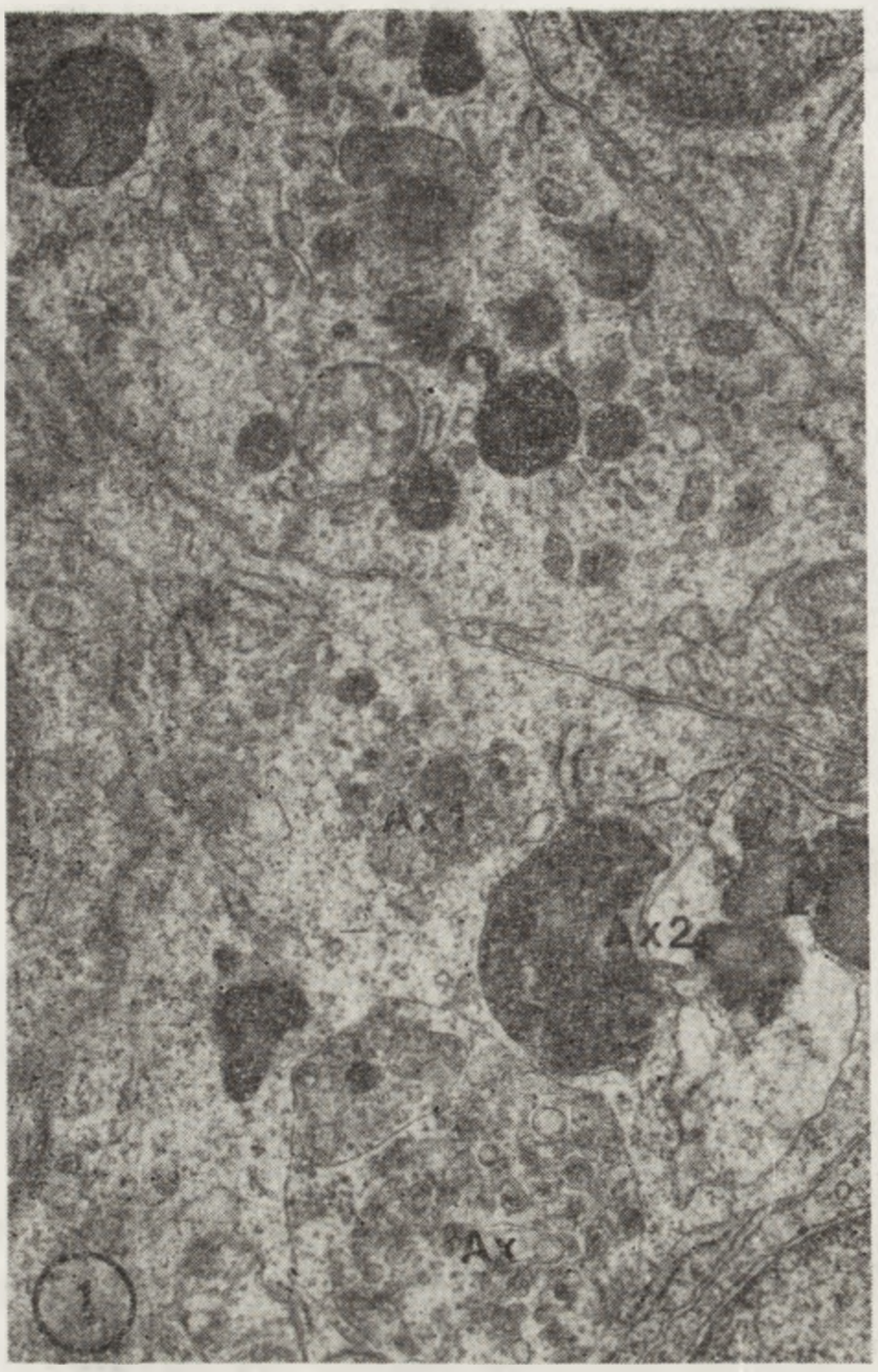
The differentiation of pituicytes is interpreted as a response to the presence of neurosecretory axons whose secretory activity is related to that of pituicytes. In the present study the fine structure of pituicytes was investigated between 1 and 30 days postnatum in organotypic neural lobe cultures with the aim of providing further insight into their role in the hypothalamo-neurohypophyseal system.

Organotypic cultures were prepared from newborn Wistar rat neural lobes, which were explanted on collagen-coated coverslips into Carrel flasks. The nutrient medium, which was renewed twice weekly, consisted of 50% human serum, 40% Earle's solution, pH 7.2, and 10% 9-day-old chicken embryo saline extract. It was supplemented with glucose to a final concentration of 600 mg/100 ml and 100 units/100 ml penicillin. The cultures were monitored in the living state by light microscopy and fixed for electron microscopy 1, 3, 7, 14, 21 and 30 days after explantation. Thin sections were examined with a JEM 7 A electron microscope.

After interraption of the connection between the hypothalamus and the neural lobe and placement of the neural lobe into tissue culture, pituicytes became phagocytic and eliminated degenerating neurosecretory axons within about two weeks after explantation. After one and three days in culture, the pituicytes appeared as a single cell type. They extended finger-like processes into the extracellular space. The cells had large, oval nuclei with marginal heterochromatin and relatively abundant cytoplasm containing prominent Golgi complexes, well developed endoplasmic reticulum, numerous ribosomes, polyribosomes and vesicles. The somata of pituicytes and their processes were in close contact with degenerating neurosecretory axons, some of which were embedded in pituicyte cytoplasm. The cytoplasm contained an increased



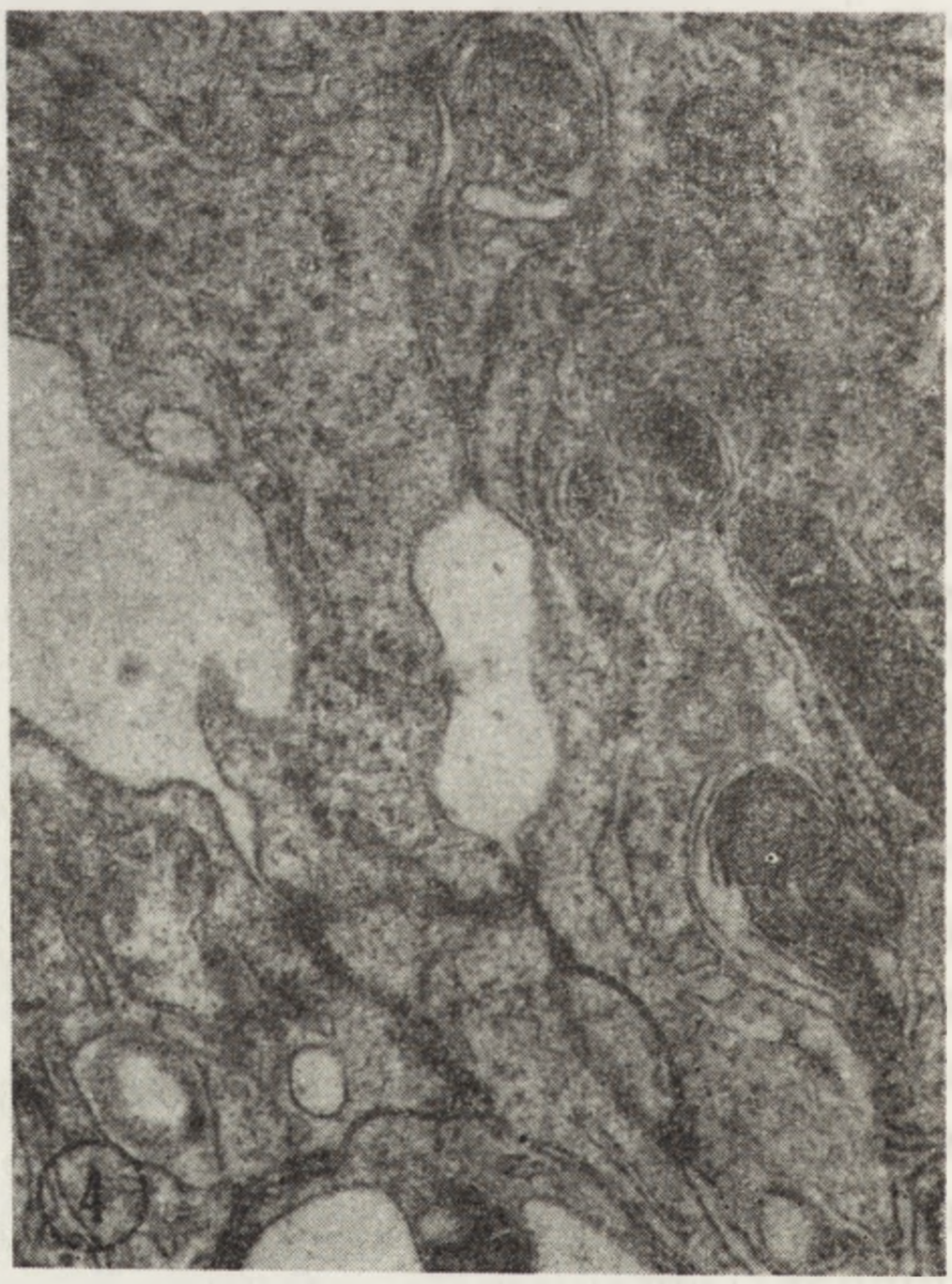


Fig. 1. Phagocytosing pituicytes of 3 day culture. Note degenerating neurosecretory axons (Ax, Ax 1 and Ax 2), lysosomes and lipid droplets (Li). × 13 500

Fig. 2. Pituicytes of 7 day culture. Cell with light cytoplasm and the process of pituicyte of dark type (P). Note glycogen in pituicyte cytoplasm. × 13 350

number of lysosomes, dense bodies, dense lamellar bodies, lipid droplets, and vacuoles containing axonal debris (Fig. 1). At seven days in vitro, two cell varieties were observed. The first, more frequent cell type was characterized by dark cytoplasm containing a large Golgi complex, numerous ribosomes, polyribosomes, ER cisternae and mitochondria. The other cell type was represented by cells with light cytoplasm containing a reduced number of most cell organelles and dark nuclei with abundant, often marginally and centrally clumped heterochromatin. Some cells showed a peculiar glycogen accumulation which was probably connected with changes in metabolic activity (Fig. 2). During further development the pituicytes enlarged and developed long, slender as well as microvilli-like processes which established a close relationship with adjacent pituicyte processes in a complex intertwining arrangement. An intercellular canalicular system appeared (Fig. 3). The cells were interconnected by numerous gap- and desmosome- type junctions. In the third and fourth weeks of cultivation the pituicytes were an intermediate type between light and dark types and had the cytological characteristics of activated cels (Figs. 3 and 4).

The observed polymorphism of pituicytes is considered to be the morphological reflection of varying activity.

Fig. 3. Pituicytes of 16 day culture. Microvilli of adjacent cells project into the extracellular space. Note well developed RER and desmosome- type junctions. × 13 350

Fig. 4. Pituicytes of 21 day culture. Note increase in the surface contact between pituicytes and intermediate junctions. × 13 350

POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (471—474)

CONDITIONAL INFLUENCE ON THE SECRETORY ACTIVITIES OF ARTERIAL SMOOTH MUSCLE CELLS

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The smooth muscle cells (SMC) are responsible for the production of proteglycans, collagen and elastic fibers during the development of arterial wall, its healing and during formation of atherosclerotic plaques. SMC have been shown to syntesize and secrete extracellular matrix components when cultured in vitro and when growing in heterotopic transplants [1, 2]. In the present study the production and secretory abilities of aortic SMC under the influence of several choosen factors were tested. The particular interest has been paid to the elastin production, because elastogenetic distribuances play the important role in pathomechanism of wide spread connective tissue diseases and arthero-sclerosis.

SMC were isolated enzymatically from the aortas of 5–8-day-old rats [2, 3] and maintained in vitro in Eagla's medium with or without 10% fetal calf serum at presence of 2% agarose, 1.0 mg% galactose or 0.1 μM dexamethasone. Isolated cells suspended in 2% agarose gel were transplanted into the back muscle of the young rats of the same strain as donor as well. Samples for light and electron microscopy were taken from cultures at 7, 14, 21 and 28 days. The transplants were dissected out after 21 days. Specimens for EM were fixed in 4% glutaraldehyde with 1% tannic acid addition, postfixed in osmium tetroxide and embedded in Spurr low viscosity resin [3]. Routinely stained thin sections were examined in a JEM-100 C EM. The parallel samples prepared for light microscopy were stained with orcein and hematoxylin. The controls with elastase digestion were also performed.

Smooth muscle cells maintained in serum supplemented medium rapidly formed confluent multiple overlaping layers surrounded by in-

Fig. 1. 14-day-old culture of SMC. Electron-dense elastin (EL) can be seen in the extracellular matrix. × 5000

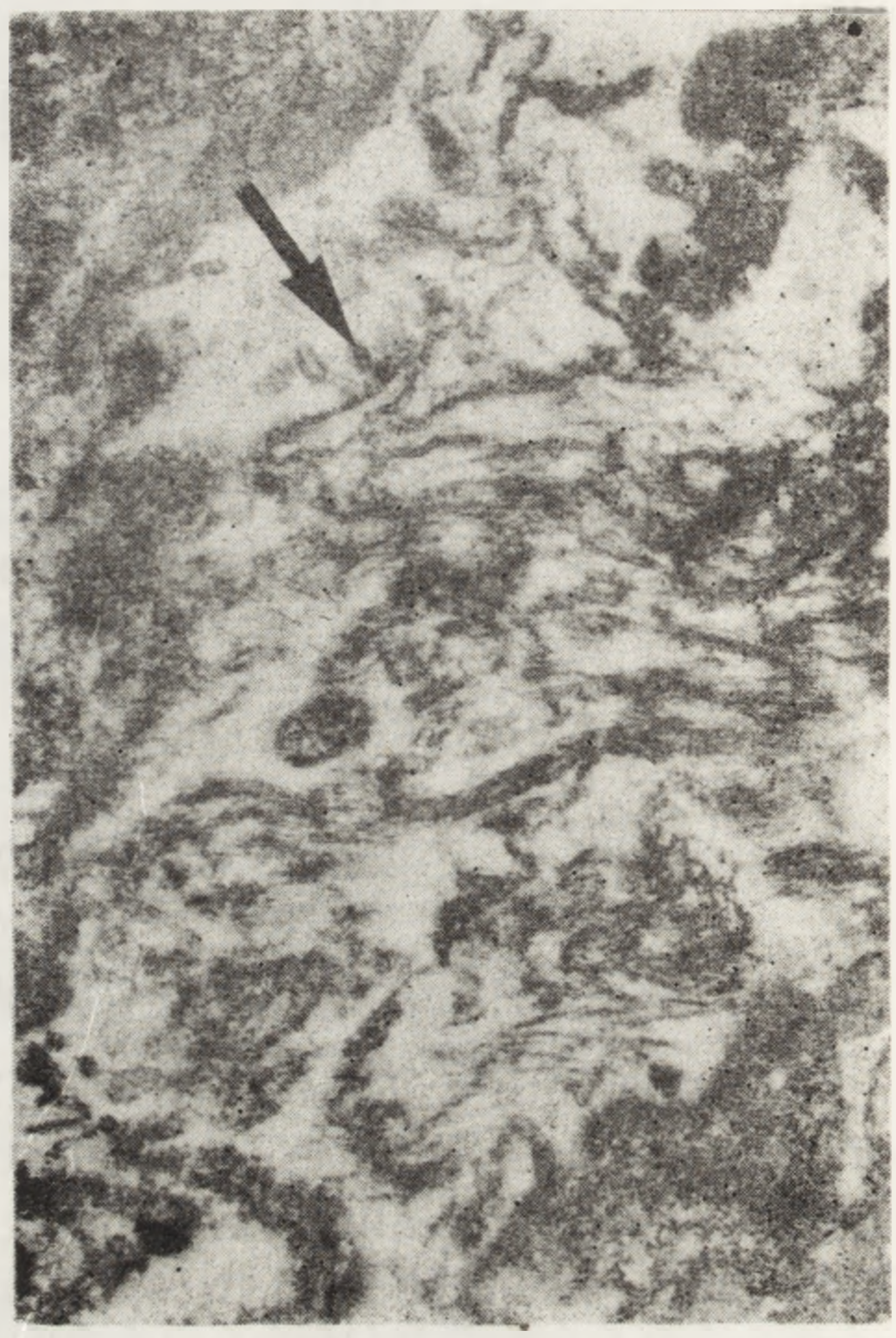

Fig. 2. 14-day-old culture of SMC covered by agarose gel. Extracellular matrix consists of glycoprotein microfibrills (arrow). × 5000

tercellular matrix consisting of collagen fibers, proteoglycans and elastic fibers of different maturity. The cells cultivated without serum were less numerous, more flated and later grew in a multilayer if compared with the cells in serum contained culture. The matrix components in such cultures were scarce. Addition of dexamethasone substantially increased the production of elastic fibers and other matrix components in both culture systems respectively, but did not influence the pattern of cell proliferation. The SMC cultivated under the agarose gel formed the matrix consisting of collagen, proteoglycans, glicoprotein microfibrills but not of elastin. The galactose increased the elastin production. The myocytes transplanted intramuscularly as a suspension in agarose gel retained their typical structure and abilities for secretion of matrix components except of elastin. This confirmed the data collected from the culture system suggesting the selective inhibition of elastogenesis by agarose. The cultured or transplanted cells did not revealed phagocytic and elastolityc activity nor the accumulation of elastin precursor in the cytoplasm. It seems that agarose may exhibit some indirect in-

Fig. 3. 14-day-old culture of SMC maintained with dexamethasone. The mature elastic fibers are evident (EL). × 5000

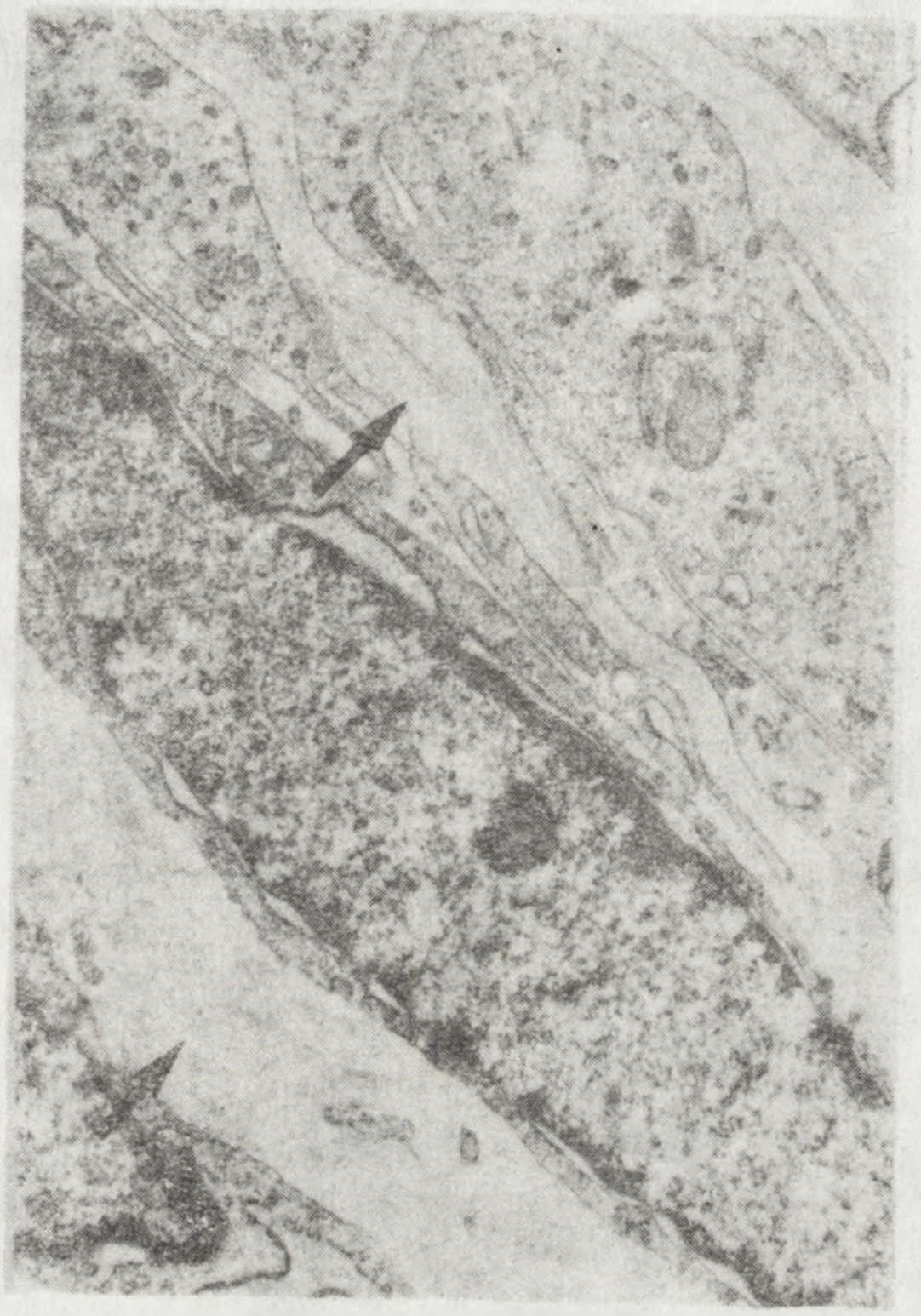

Fig. 4. 21-day-old transplant of SMC in agarose. SMC are surrounded by extracellular matrix consisting of glycoprotein microfibrills and collagen fibers (arrows). × 5000

fluence for the genetic expresion of SMC induced by contact with the cell surface. The inhibition of elastogenesis does not depend of absorption on the agarose of any possible elastogenic inductors from the serum and is reversible after agarose removing. The nature of postulated intracellular signal of messenger induced by the contact of agarose with the cell surface needs further elucidation.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (475—478)

ULTRASTRUCTURAL INVESTIGATION OF MITOCHONDRIA OF HEPATOCYTES FROM DIFFERENT ZONES OF LOBULI IN CIRCADIAN RHYTHM

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Metabolic activity of different organs undergoes some cyclic changes according to both circadian and seasonal rhythms. Circadian changes of some processes in liver have been reported, some of these changes can affect the ultrastructure of hepatocytes and they can be followed using EM methods. In spite of anatomical homogeneity one can distinguish three different ultrastructural and functional zones in every hepatic lobule, i.e. peripheral, midzonal and central [3]. The aim of our work was to establish the stereological parameters of mitochondria from central and peripheral zones of hepatic lobules taken at the chosen time of the day. The experiments were performed with Wistar male rats in May after the synchronization of light conditions LD 12 : 12 during two weeks. The animals were killed at 8 a.m. and 10 p.m. These times were chosen as they were reported to be the times of maximal and minimal glycogene contens in rat liver in May [4, 5]. The stereological analysis of sections was carried out on central and peripheral parts of hepatic lobule, according to Weibel [6]. Surface density and volume density of both inner and outer membranes and compartments were evaluated. The calculations including the statistic analysis were performed using ODRA 1305 computer using tests of *t*-Student, as well as those Satterwhite and Willcoxon.

The visual estimation of electronomograms does not reveal any changes. However, the statistic analysis of stereological data shows us the significant differences between the mitochondria of the central and those of the peripheral zones (Tab. 1). Mitochondria of peripheral zone can be distinguished by their smaller volume density of matrix (V_{ic}) as

TABLE 1

Morphometric characteristic of the investigated mitochondria

Compartment	Parameter	Symbol	Dimension	Peripheral cells 8 a.m.	Peripheral cells 10 p.m.	Central cells 8 a.m.	Central cells 10 p.m.
Outer membrane	Surface	S_{om}	$\mu m^2/\mu m^3$	6,25 ± 0,24	5,33 ± 0,25	5,59 ± 0,26	6,24 ± 0,27
	Volume	V_{om}	%	3,32 ± 0,39	3,42 ± 0,27	2,81 ± 0,22	2,59 0,28
Inner membrane	Surface	S_{im}	$\mu m^2/\mu m^3$	13,99 ± 0,52	13,70 ± 0,58	13,71 ± 0,56	15,27 ± 0,64
	Volume	V_{im}	%	6,05 ± 0,41	5,52 ± 0,34	4,98 ± 0,28	5,26 ± 0,37
Inner mem./ Outer membrane	Surface	S_{im}/S_{om}	–	2,36 ± 0,10	3,11 ± 0,38	2,66 ± 0,11	2,66 ± 0,16
Outer compartment	Volume	V_{oc}	%	8,96 ± 0,46	8,29 ± 0,52	8,22 ± 0,51	9,11 ± 0,44
Inner compartment	Volume	V_{ic}	%	81,66 ± 0,68	82,77 ± 0,69	83,99 ± 0,67	83,04 ± 0,55
Outer comp. Inner membrane	Partition coefficient	E_{ocm}	$\mu m^3/\mu m^2$	0,064 ± 0,003	0,053 ± 0,003	0,068 ± 0,025	0,060 ± 0,023
Inner comp./ Inner membrane	Partition coefficient	E_{mm}	$\mu m^3/\mu m^2$	0,0071 ± 0,0004	0,0064 ± 0,0004	0,0061 ± 0,0004	0,0065 ± 0,0004

All parameters are expressed per μm^3 or per cent mitochondrial volume excluding partition coefficient and represent the mean ± standard error.

well as the increase in the volume density and surface of outer membrane (S_{om}, V_{om}). However the partition coefficient E_{mm} (the ratio of matrix volume to inner membrane surface) in peripheral zone is significantly increased. The volume density of the outer mitochondrial compartment (V_{oc}) in peripheral zone is significantly lower at 10 p.m. when compared to that at 8 a.m. At the some time the surface density of outer membrane (S_{om}) is decreased. So is the ratio of surface of inner to outer membrane (S_{im}/S_{om}). Surface density of both outer and inner membrane (S_{om}, S_{im}) of mitochondria of the central zone is higher at 10 p.m. than at 8 a.m. The coefficient E_{mm} is higher, too. Taking into consideration the relationship between the inner membrane configuration and metabolic activity of mitochondria [1, 2] we can draw the following conclusion: liver mitochondria in central zone of lobuli revealed a circadian rhythm with tendency of these mitochondria to change their state from the typical orthodox to the intermediate state at 8 a.m.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (479—484)

EVALUATION OF DISTRIBUTION OF DIAMETERS OF VESICLES FROM THE ISOLATED SUBCELLULAR FRACTIONS OF ROUGH (RER = R) AND SMOOTH ENDOPLASMIC RETICULUM (SER = S) OF NORMAL AND REGENERATING RAT LIVER

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Bont [1] has shown that all the vesicles, both obtained during the homogenization of membranes and those naturally occurring within the cells, are restricted to certain dimensions. Their diameters must be the consecutive terms of two geometric series. Anotherwords, some dimensions are not permitted. Among the others it concerns the granules containing pituitary hormones and synaptic vesicles. Two geometric series,

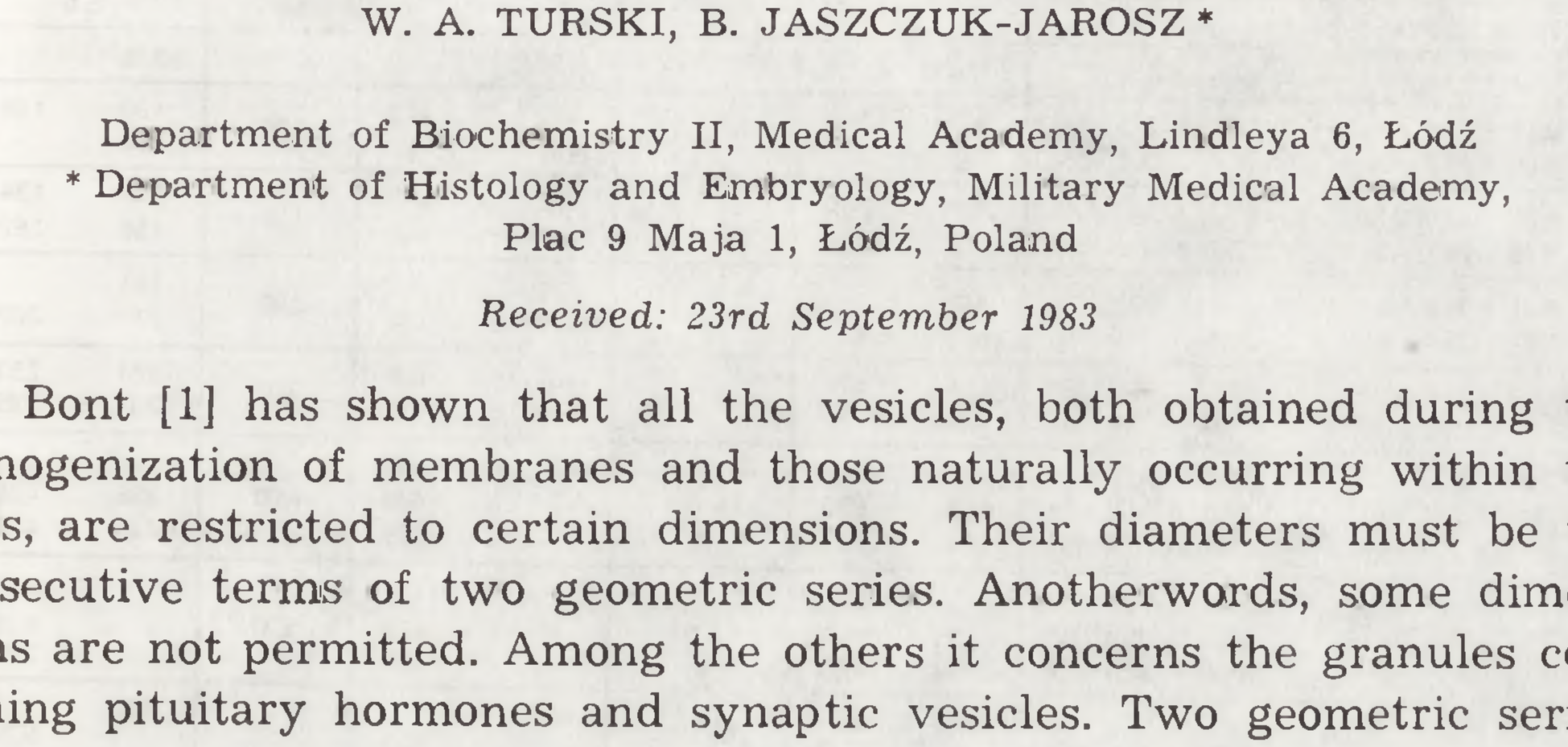

Fig. 1. The histogram of diameters of rough (*R*) and smooth (*S*) vesicles (from control rats). On the abscissa: diameters of vesicles (in nm); on the oridinate: the number of vesicles within given limits of diameters

mentioned above, may result from the fusion of "unit" vesicles and next the identical vesicles, except that the "dimer" vesicle ($V = 2$) may fuse with the "unit" one giving the "trimer" vesicle ($V = 3$). So, the V values in two series are 2, 4, 8, 16, 32, etc. in II and 3, 6, 12, 24, 48, etc. in I of series of Bont.

The diameters fit the formula:

$$D(V) = [D(1) - 100]\sqrt{V} + 100.$$

/diameter/ D /Å/ ± σ /number of vesicles/		V calculated assuming D(1) = 433 A		V /serie of Bont/	D/1/ calculated		Mass ratio theo - vetical	observed	
smooth /351/	rough /387/	smooth	rough		smooth	rough		smooth	rough
1		2		3	4		5a	5b	
1263 ± 39.2 /24/		12.2		12 /I/	436		0.75	0.76	
1433 ± 28.4 /36/	1480 ± 21.2 /3/	16.0	17.2	16 /II/	433	445	1.00	1.00	1.08
1545 ± 25.0 /33/		18.8			461			1.18	
1627 ± 32.0 /27/	1642 ± 44.9 /18/	21.0	21.4	24 /I/	412	415	1.50	1.71	1.34
1773 ± 29.1 /48/	1793 ± 35.6 /54/	24.9	25.9		440	446		1.56	1.62
1941 ± 34.6 /42/		30.6		32 /II/	425		2.00	1.91	
2061 ± 34.8 /36/	2015 ± 55.0 /66/	34.7	33.1		447	439		2.17	2.07
2209 ± 30.8 /21/	2217 ± 36.0 /36/	40.1	40.4	48 /I/	404	406	3.00	2.51	2.53
2373 ± 63.3 /48/	2348 ± 13.6 /36/	46.6	45.6		428	425		2.91	2.85
	2559 ± 41.9 /42/		54.5			407			3.41
2718 ± 46.2 /18/	2788 ± 61.6 /48/	61.8	65.2	64 /III/	427	436	4.00	3.86	4.08
2913 ± 47.3 /9/	2998 ± 31.9 /15/	71.4	75.7		452	462		4.46	4.73
	3144 ± 28.8 /21/		83.6			411			5.23
	3411 ± 75.9 /24/		98.9	96 /I/		438	6.00		6.18
	3640 ± 82.0 / 3/		113.0			461			7.06
3993 ± 63.5 /9/		136.0		128 /II/	444		8.00	8.50	
	4150 ± 14.1 / 6/		147.9			458			9.24
	4495 ± 133.0 /12/		174.2	192 /I/		417	12.00		10.89
	6400 ± 58.0 / 3/		357.9	384 /I/		422	24.00		22.37

Fig. 2. (table) Analysis of diameters of rough (R) and smooth (S) vesicles (from control rats). **Column 1:** σ — standard deviation; in parentheses: number of vesicles. **Column 2:** the V value, i.e. the number of "unit" vesicles per 1 "real" vesicle was calculated from $D(V)$ values from column 1, according to the formula: $V = \left[\frac{D(V)-100}{D(1)-100}\right]^2$ of Bont [1] assuming $D(1)$, i.e. the diameter of "unit" vesicle, as 433 Å as was calculated by Bont [1]. **Column 3:** "permitted" V value being the nearest to the real values given in column 2. Number of series according to Bont [1] is given in parentheses. **Column 4:** D(1) was calculated according to the formula: $D(1) = \frac{D(V)-100}{\sqrt{V}} + 100$ of Bont [1]; where $D(V)$ are the real mean diameters (see column 1) for V values shown in column 3. **Column 5 a and 5 b** give us the mass ratio, i.e. **5a:** theoretical being the ratio of respective V shown in column 3 and 5b: observed mass ratio calculated as $\left[\frac{D(V)-100}{D(16)-100}\right]^2$ where $D(16)$ is 1433 Å (see column 1)

We decided to evaluate the distribution of diameters of vesicles from the isolated subcellular fractions R and S from normal and regenerating rat liver.

Fractions R and S were obtained and analyzed as we described [2]. The preparation of ultrathin sections of pellets of fractions to be screen using a Phillips EM 300 device was described elsewhere [3].

Diameters of vesicles were measured directly on EM pictures under different magnification from 13 000 to 25 000 (additional photographic magnification × 3) with the ordinary measure with the accuracy ±0.5

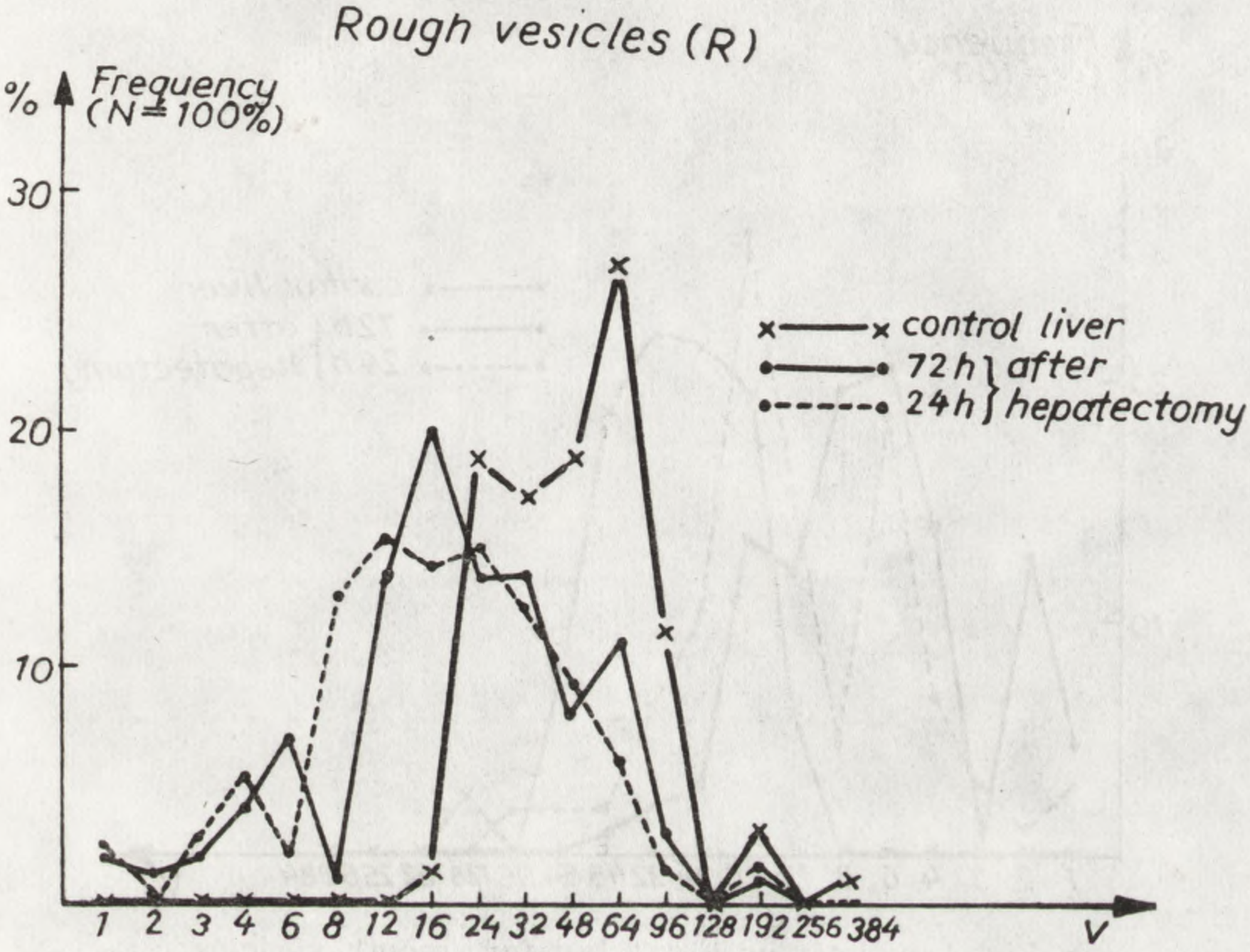

Fig. 3. The histogram of frequency of rough vesicles of given V values (compare text and Fig. 2) from control liver and from liver regenerating 24 and 72 hours after partial hepatectomy. Frequency was calculated as

$$\frac{\text{number of vesicles of given } V}{\text{total number of vesicles}} \cdot 100\%$$

mm. The measure was standarized by comparison with the lines measured with the micrometer screw.

The EM pictures of fraction S and R are presented elsewhere [4, 5]. The vesicles are mostly spherical. The percentage of those elliptic is hardly more than 15%. As we can see (Fig. 1) the distribution of diameters neither R nor S vesicles is Gaussian one (or any other "normal"). A lot of values seems to be favoured, while many other seem to be "prohibited". Indeed, the permitted diameters (Fig. 2) show the negligible standard deviation from mean values which in turn fit to

two geometric series producted by Bont [1]. The diameter of the "unit" vesicle calculated with the forementioned formula is always almost the same, i.e. 436 ± 14 (Å) and the same as calculated by Bont from sedimentation constants of vesicles, i.e. 433 ± 12 [1]. Mass ratio of vesicles which is calculated from experimental data is identical within ± 15% range with the theoretical one. Some discrepancies of diameters of given *V* values seem to come rather from small deviations from closely spherical form than from the heterogeneity of vesicles themselves.

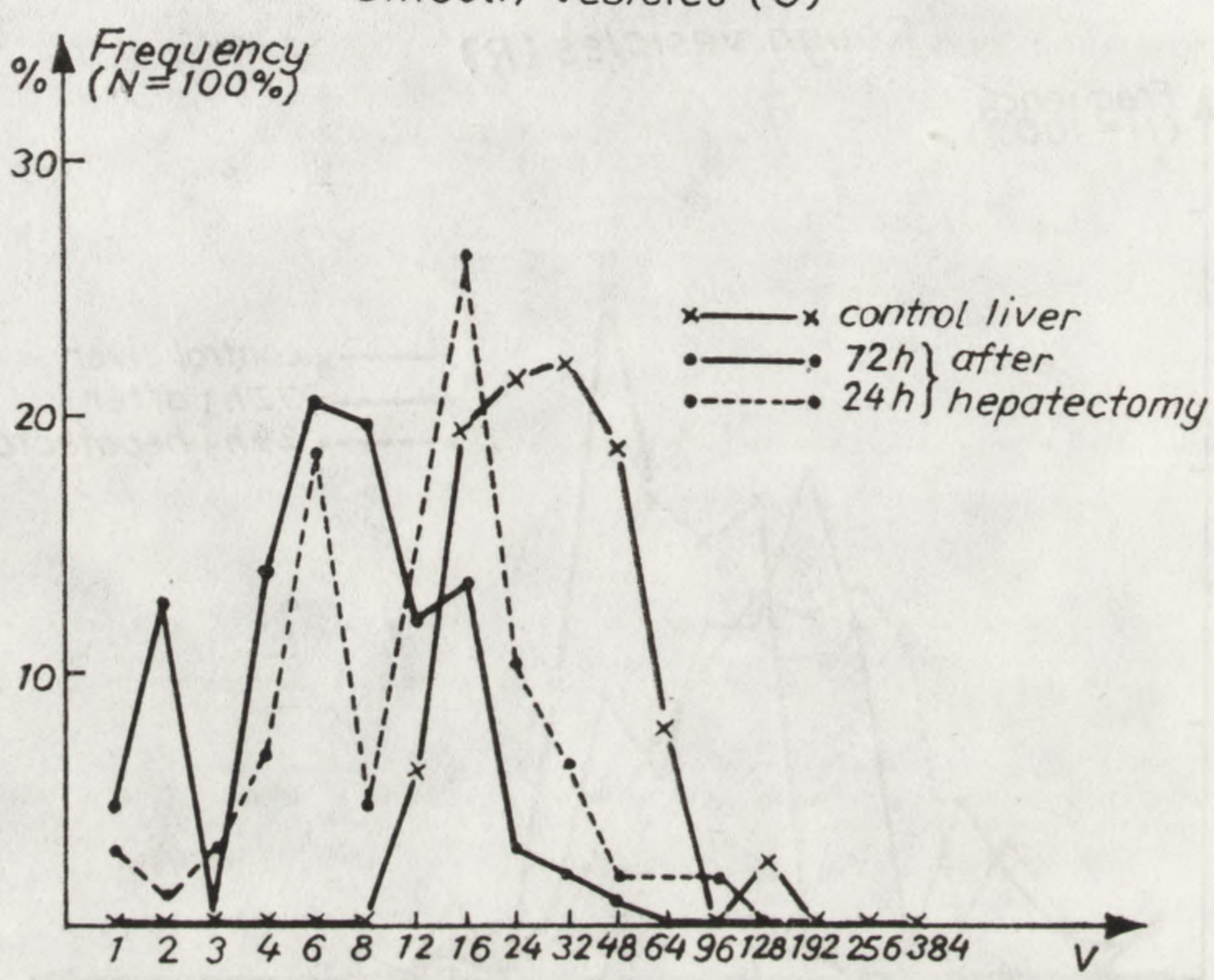

Fig. 4. exactly as Fig. 3 but for smooth vesicles

The best confirmation of Bont theory are the largest vesicles, which even when rare never exist when of not permitted *V* and *D* value. The frequency of terms of both series of Bont: II and I is about the same. So are the diameters of vesicles coming from rough and smooth ER (having the same *V* value). The pattern of R vesicles is always shifted to higher *V* values when compared with S vesicles.

Fig. 3 shows the pattern of frequency of different V values for R vesicles, while Fig. 4 the same for S vesicles. As we can see 24 h after partial hepatectomy there is a lot of very small vesicles (*V* from 1 to 12) which are not observed in control liver (both Fig. 3 and 4), while the percentage of bigger vesicles remarkably decreases. As the vesicles of R and S result from fragmentation of RER and SER it looks like the newly formed membranes of both rough and smooth reticulum be-

come more fragile than preexisting ones. The opposite direction of changes of diameters of R and S vesicles between 24 and 72 h after hepatectomy (Fig. 3 and 4) can be revealed. While the rough vesicles seem to normalize quite a bit, those of SER stay in the same range or even decrease. It means that normalization of fragility of rough membranes is close to the end after 72 h, while in smooth membranes the normalization is far to come as the more fragile newly formed pieces of SER membranes still appear.

It seems certain that besides the unique places active in fusion process of "unit" (and respective larger) vesicles there must be, may be the same, unique places of splitting of membranes surface. At the moment, no one can tell anything about the characteristic of "fragile places". It might be the biogenesis of membranes goes through the addition of the blocks with about the same mass and composition as the "unit" vesicles of Bont to the preexisting membranes.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (485—486)

INFLUENCE OF ANALOGS OF (SP_{5-11} AND SP_{6-11}) SUBSTANCE P FRAGMENT ON THE MONOAMINE OXIDASE ACTIVITY IN VARIOUS AREAS OF THE RAT BRAIN

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In this study we examined the in vitro influence of substance P fragment analogs on the activity of monoamine oxidase (MAO) in homogenates and crude mitochondrial fractions of the six areas of rat brain: I — cerebral cortex, II — hippocampus, III — midbrain, IV — thalamus with hypothalamus, V — cerebellum and VI — medulla oblongata. The obtained results prove that the analogs of substance P fragments that we applied inhibit selectively the activity of the enzyme

TABLE 1

The influence of SP_{5-11} analog on the activity of MAO from six areas of rat brain

Brain area		Control without (SP_{5-11})	With SP_{5-11}	% of decrease	% of increase
I	H	0.1701	0.1315	22.7	—
	M	0.0490	0.0626	—	27.8
II	H	0.1930	0.1033	46.5	—
	M	0.0598	0.0752	—	25.7
III	H	0.1310	0.1140	13.0	—
	M	0.0766	0,0762	—	—
IV	H	0.1402	0.1454	—	3.7
	M	0.0930	0,0700	24.7	—
V	H	0.1796	0.1267	29.5	—
	M	0.0817	0.0909	—	11.3
VI	H	0.0926	0.0966	—	4.3
	M	0.0607	0.0517	14.8	—

H — homogenate, M — mitochondria

TABLE 2

The influence of SP_{6-11} analog on the activity of MAO from six areas of rat brain

Brain area		Control without SP_{6-11}	With SP_{6-11}	% of decrease	% of increase
I	H	0.1788	0.1155	35.4	—
	M	0.0490	0.0648	—	32.2
II	H	0.1324	0.0545	58.5	—
	M	0.0603	0.0759	—	25.9
III	H	0.1680	0.1475	12.2	—
	M	0.1122	0.1132	—	—
IV	H	0.1402	0.1361	2.9	—
	M	0.1181	0,0990	16.2	—
V	H	0.2229	0.1605	28.0	—
	M	0.0799	0.0838	—	4.9
VI	H	0.0966	0.1042	—	7.9
	M	0.0449	0.0335	25.4	—

H — homogenate, M — mitochondria

in the homogenates of the cerebral cortex, hippocampus, midbrain, cerebellum and in mitochondrial fraction of thalamus with hypothalamus and medulla oblongata. In the mitochondrial fraction of midbrain the applied analogs have no influence on the enzyme activity. The most significant changes in monoamine oxidase activity (a 50% decrease) were observed in hippocampus homogenate fraction.

TABLE 3

Protein content in the rat brain (in mg protein/g wet weight tissue)

I	H	136.9
	M	64.8
II	H	267.2
	M	146.2
III	H	212.2
	M	108.8
IV	H	245.7
	M	122.4
V	H	283.4
	M	129.8
VI	H	360.0
	M	162.0

POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (487—490)

ACTION OF Ca AND SUBSTANCE P ON THE (Ca, Mg) ATPase ACTIVITY OF SYNAPTOSOMES OF DIFFERENT AREAS OF RAT BRAIN

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It is suggested that the transmitter release is controlled mainly by depolarization of the presynaptic neurons and by the amount of calcium which subsequently enters the synaptic terminals. Thus, the limited influx of Ca^{2+} may cause a reduction in the release of neurotransmitters.

It is known that substance P (SP) exerts strong depolarization of motoneurons and decreases ionic conductance.

Considering the fact that the distribution of Ca is connected with the activity and localization of brain membrane (Ca, Mg) ATPase (E.C.3.6.1.3), being responsible for the active transport of Ca^{2+}, it seems to be of interest to find the relationship between the activity of (Ca, Mg) ATPase and various concentrations of Ca^{2+} and SP.

Adult male Wistar rat brains (200–250 g) were divided on ice into four areas: I — cerebral cortex, II — hippocampus, III — midbrain and IV — thalamus with hypothalamus.

The crude synaptosomal fractions were obtained according to the method of Cotman et al. The quantitative and qualitative control of the preparation was made by electron microscopy.

The incubation of synaptosome fractions with 10 μM SP or with several concentrations of $CaCl_2$ (0.01–0.8 mM of Ca^{2+}) were performed at 30°C for 20 minutes. Activity of (Ca, Mg) ATPase was determined by the coupled enzyme assay, according to Weidekam et al., and the results were expressed in μmoles P_i/min/mg protein. Protein was measured by the Lowry's method, using bovine serum albumin as standard. Experimental points are the averages of five determinations each performed in duplicate.

The substance P in vitro causes the increase of (Ca, Mg) ATPase activity in synaptosomes of hippocampus, midbrain and thalamus with

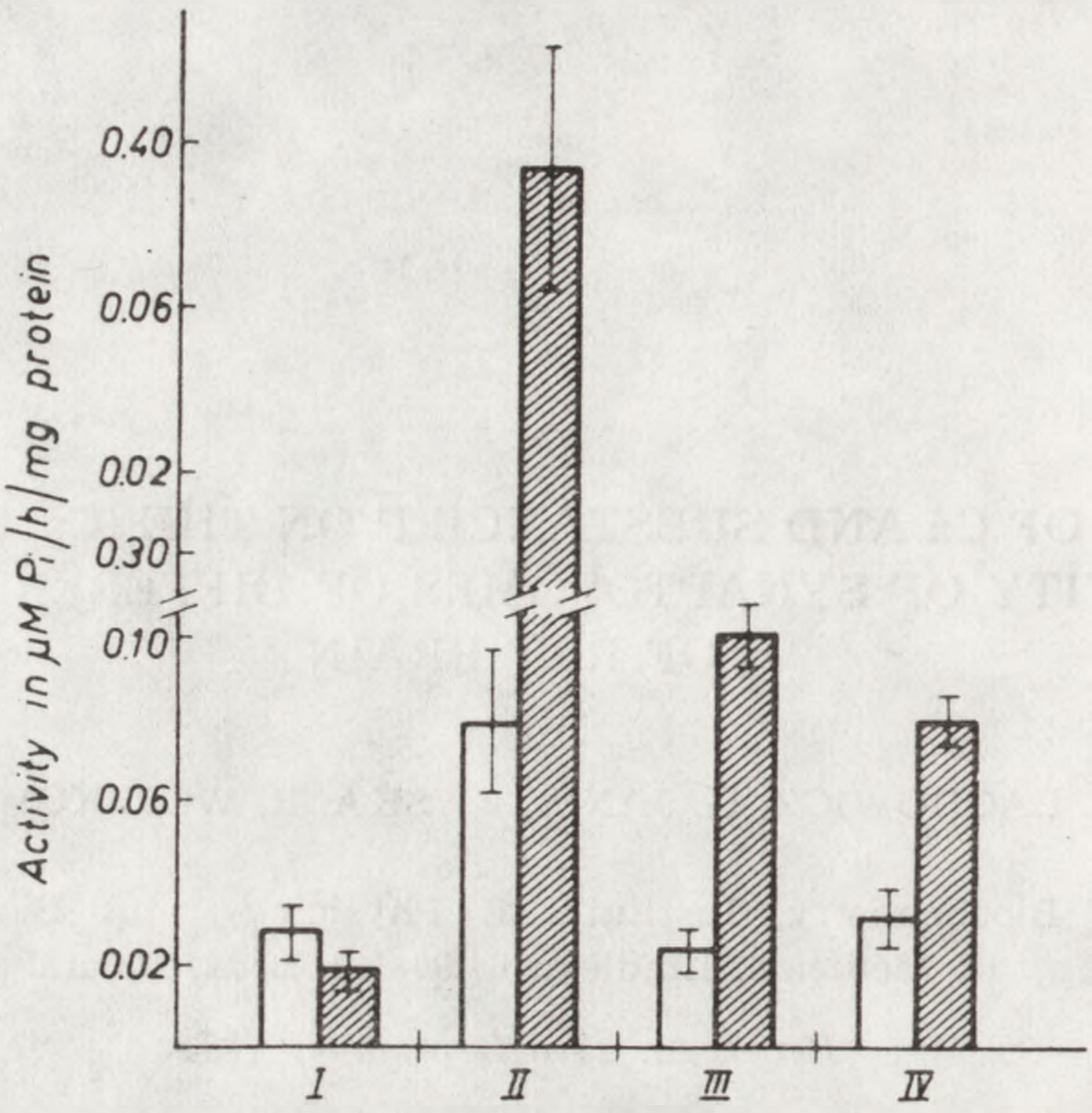

Fig. 1. Effect of SP on (Ca, Mg) ATPase activity of synaptosomes from different areas of rat brain; I — cerebral cortex, II — hippocampus, III — midbrain, IV — thalamus with hypothalamus. Activity in the presence of 2×10^{-4} M Ca^{2+} (□), and in the presence of 2×10^{-4} M Ca^{2+} and 10 μM SP (■)

hypothalamus, and the decrease of the activity in synaptosomes of cerebral cortex (Fig. 1).

Figures 2, 3, 4 and 5 indicate the influence of various concentrations of calcium (0.01–0.8 mM) on the (Ca, Mg) ATPase activity. It has

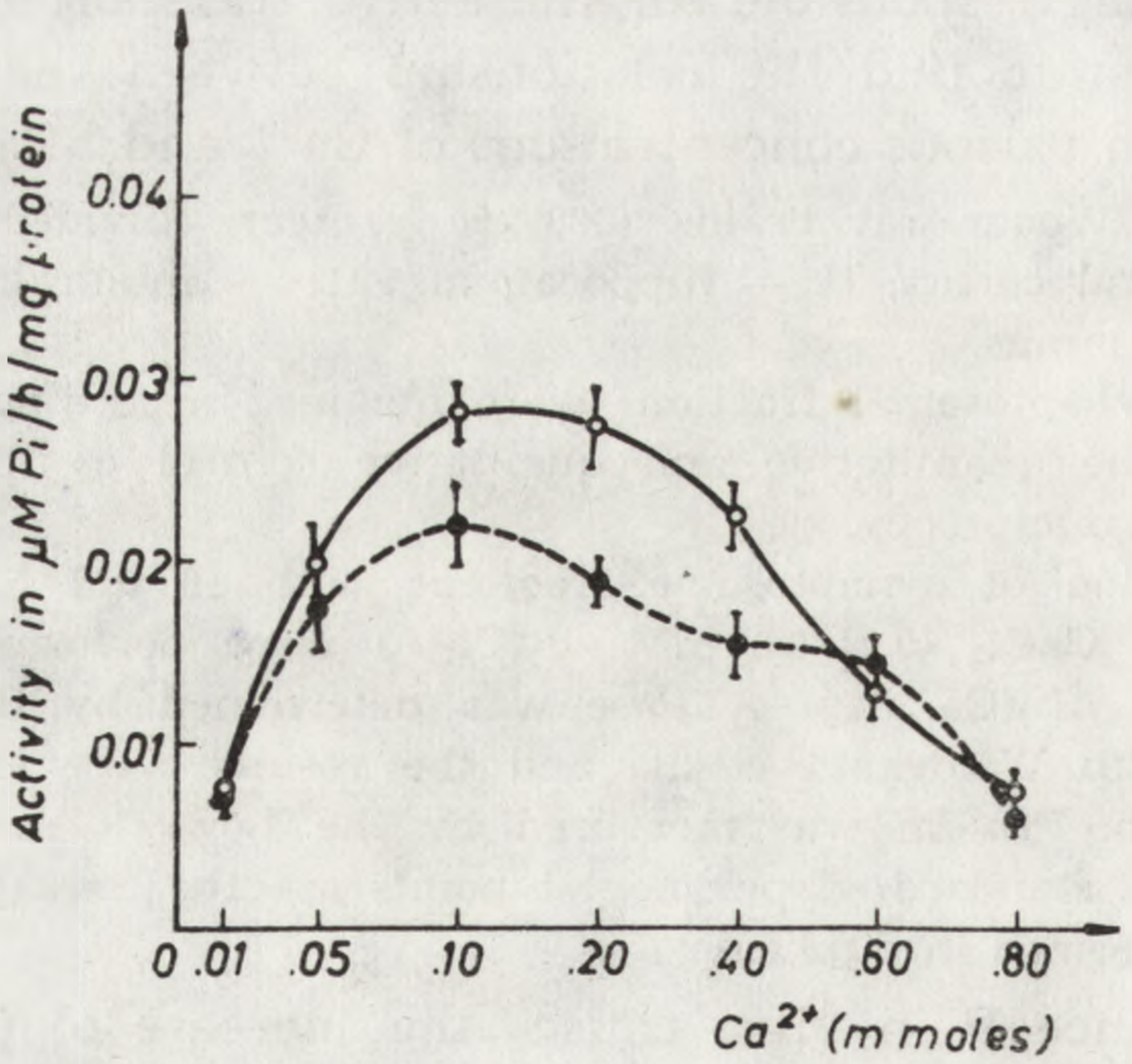

Fig. 2. Activity of (Ca, Mg) ATPase cerebral cortex synaptosomes in the presence of various Ca^{2+} concentrations — o — o, and additionally 10 μm SP – ● – ●

been confirmed that the highest activity of (Ca, Mg) ATPase appears when the calcium concentration is between 0.1 and 0.2 mM.

Concentration of Ca^{2+} below 0.1 mM and above 0.2 mM causes the reduction of (Ca, Mg) ATPase activity of synaptosomes in all the examined regions of the brain. We determined the influence of exogenous SP on the activity of this enzyme, when various amounts of calcium were used (Figs. 2–5).

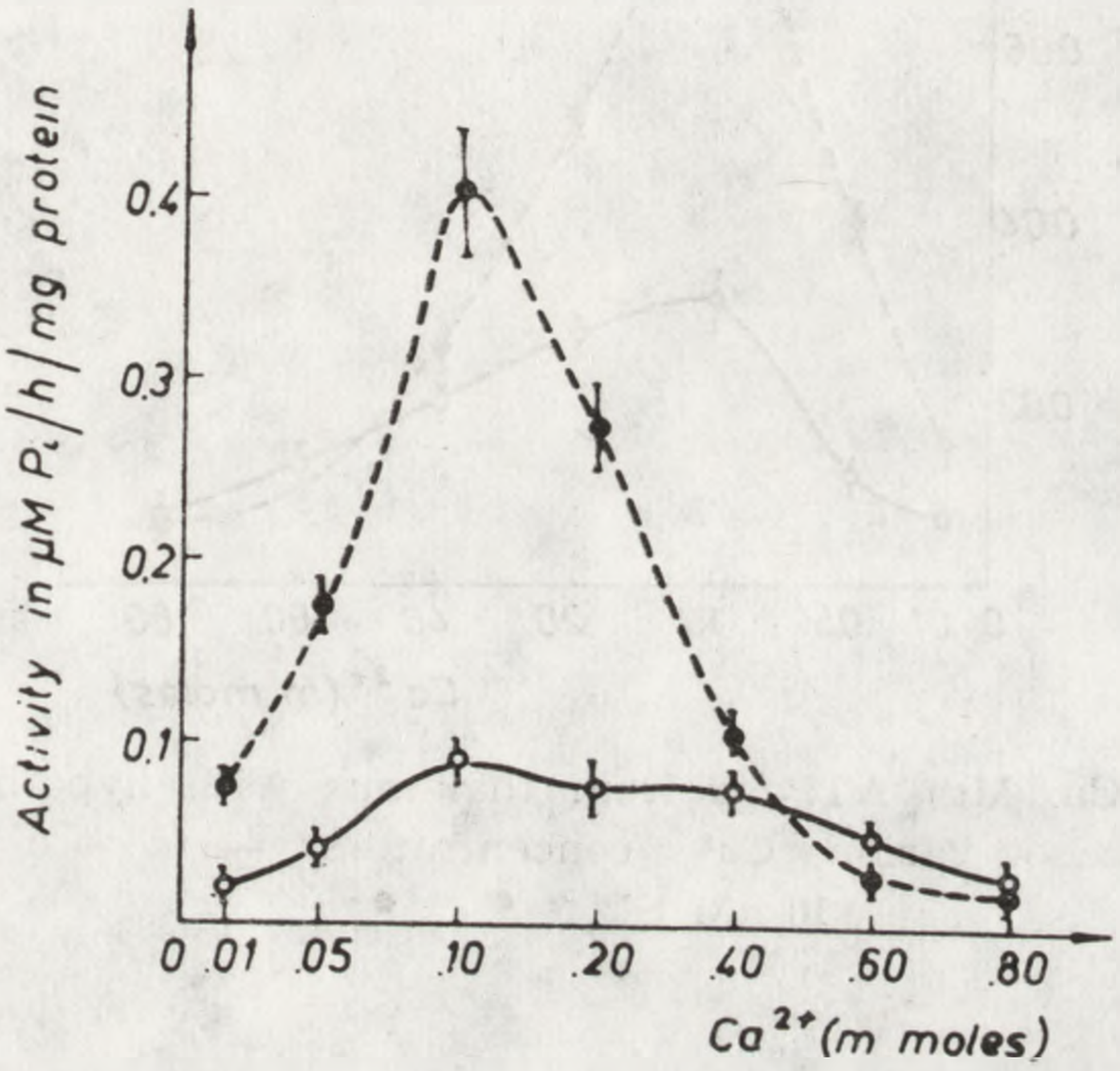

Fig. 3. Activity of (Ca, Mg) ATPase from hippocampus synaptosomes in the presence of various Ca^{2+} concentration — o — o, and additionally 10 μM SP – ● – ●

The inhibitory or stimulatory action of SP on the activity of (Ca, Mg) ATPase is most significant when the Ca^{2+} concentration in medium is within 0.1 mM and 0.2 mM. We found that both too low and too high Ca^{2+} concentrations disturb the activating effect of SP on the enzyme.

Our results can contribute to the explanation of the mechanism regulating many processes depending upon Ca^{2+} concentration, and of SP action on the activity of (Ca, Mg) ATPase in synaptosomes of hippocampus, midbrain and thalamus with hypothalamus.

These results confirm the studies of Bender et al. (1980), showing that the elimination of Ca^{2+} from the medium decreases the uptake the transmitter such as adenosine in synaptosomes. General experimental conclusion of this work points to the existence of synergistic effect of Ca and SP action in vitro on the activity of (Ca, Mg) ATPase of synaptosomes, but only at certain calcium concentration.

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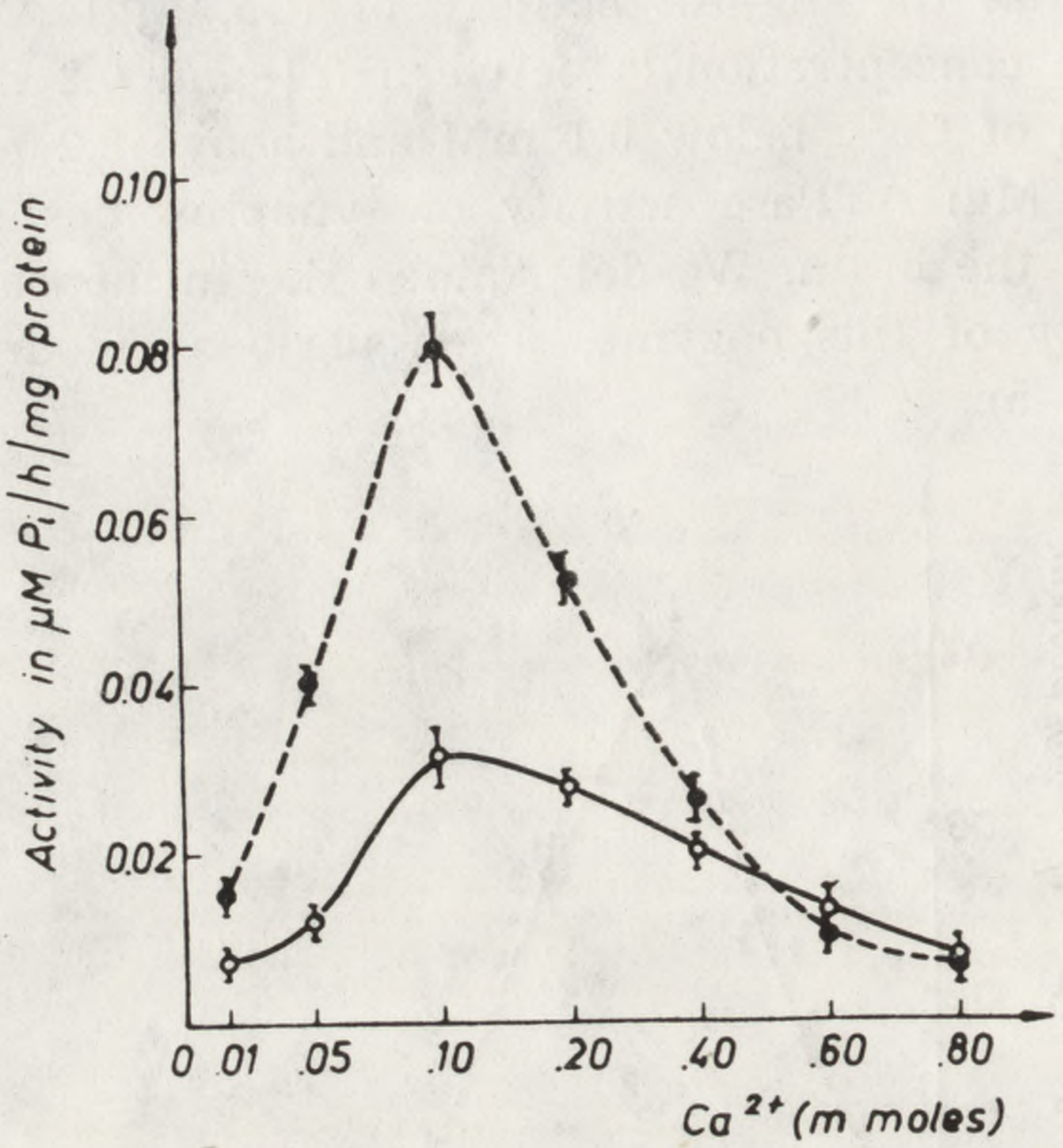

Fig. 4. Activity of (Ca, Mg) ATPase from thalamus with hypothalamus synaptosomes in the presence of various Ca^{2+} concentrations — o — o, and additionally 10 μM SP – ● – ●

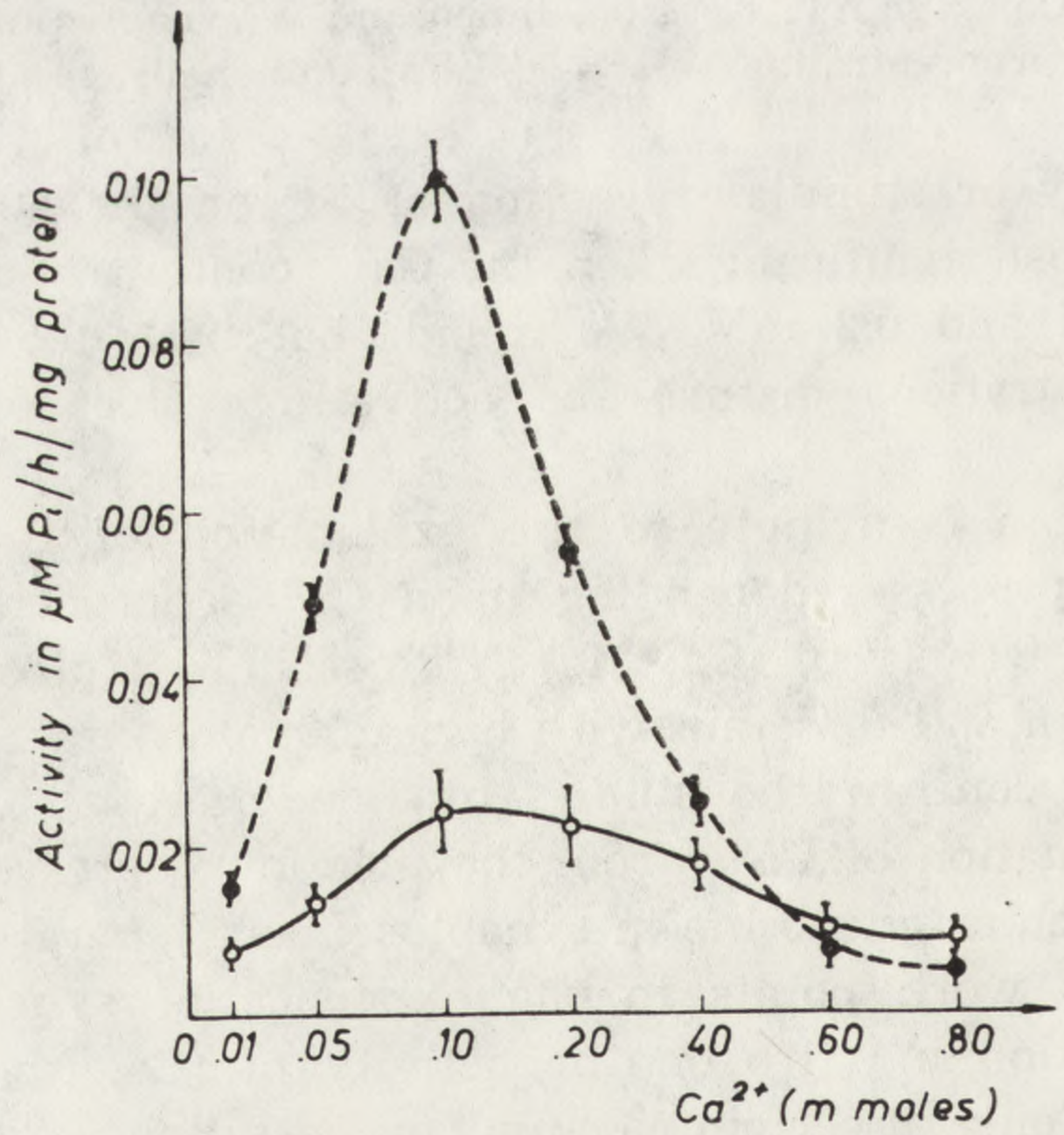

Fig. 5. Activity of (Ca, Mg) ATPase from midbrain synaptosomes in the presence of various Ca^{2+} concentrations — o — o, and additionally 10 μM SP – ● – ●

POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (491—494)

INFLUENCE OF N-ETHYL-N-NITROSOUREA UPON THE ENDOPLASMIC RETICULUM AND THE ACTIVITY OF CYTOCHROME P-450-DEPENDENT MONOOXYGENASES IN THE HEPATOCYTES

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Carcinogenic compounds of N-nitrosourea group are able to bound firmly to nucleic acids. These compounds are biotransformated in the living organism by cytochrome P-450-dependent monooxygenases. The aim of the paper was to determine the activity of mixed-function oxidases as well as ultrastructure of the hepatocytes after treatment with a single toxic dose of N-ethyl-N-nitrosourea (ENU).

Adult male Wistar rats (70 animals) were used for the experiment. Experimental group of animals was given 200 mg/kg of body wt. of ENU i.p. Rats were killed by decapitation 1, 3, 6, 12, 24 and 120 hrs after ENU injection. Control group of animals was given injection of the similar amount of physiological saline.

Liver microsomal fraction was obtained according to Dallner [1]. The activity of cytochrome P-450 and cytochrome b_5 [2], NADPH-cytochrome c reductase and NADH-cytochrome b_5 reductase [3], aniline hydroxylase [4], 4-aminopyrine demethylase [5] and protein content [6] were determined in the microsomal fraction.

Liver for ultrastructural investigations was fixed with glutaraldehyde-perfusion in situ. Ultrathin sections were stained with lead citrate and uranyl acetate according to routine procedure.

Trends of all studied enzyme changes were similar after ENU injection (Table 1). The decrease of the enzyme activities was observed 1hr after the injection. An increased values were found 3–6 hrs after the injections, and were followed by the activity decrease. Normal values of cytochrome P-450 and other enzymes activity were observed 24 hrs and 120 hrs after the injection respectively. Changes of the activ-

TABLE 1

Activity of cytochrome P-450-dependent monooxygenase and other related enzymes in the liver microsomal fractions of rats intoxicated with a single dose of ENU

Specification	Enzymatic activity (relative values; control group = 100)					
	Time after ENU injection (hrs)					
	1	3	6	12	24	120
P-450*	97.7	114.1	164.9	145.2	107.8	110.7
b_5	83.7	96.4	132.4	127.4	87.1	82.2
NADPH	92.2	108.7	135.9	100.0	72.8	114.6
NADH	80.2	109.0	124.9	101.1	86.7	97.5
HA	38.8	45.3	84.2	63.4	36.0	107.7
DA	53.4	55.7	64.2	45.7	24.5	93.9

* P-450 — cytochrome P-450; b_5 — cytochrome b_5; NADPH — NADPH-cytochrome c reductase; NADH — NADH-cytochrome b_5 reductase; HA — aniline hydroxylase; DA — 4-aminopyrine demethylase

ity of aniline hydroxylase and 4-aminopyrine demethylase were most evident. The decrease of these enzymes activity 1hr after injection was very high, and normal values were not found even 6hrs after ENU treatment.

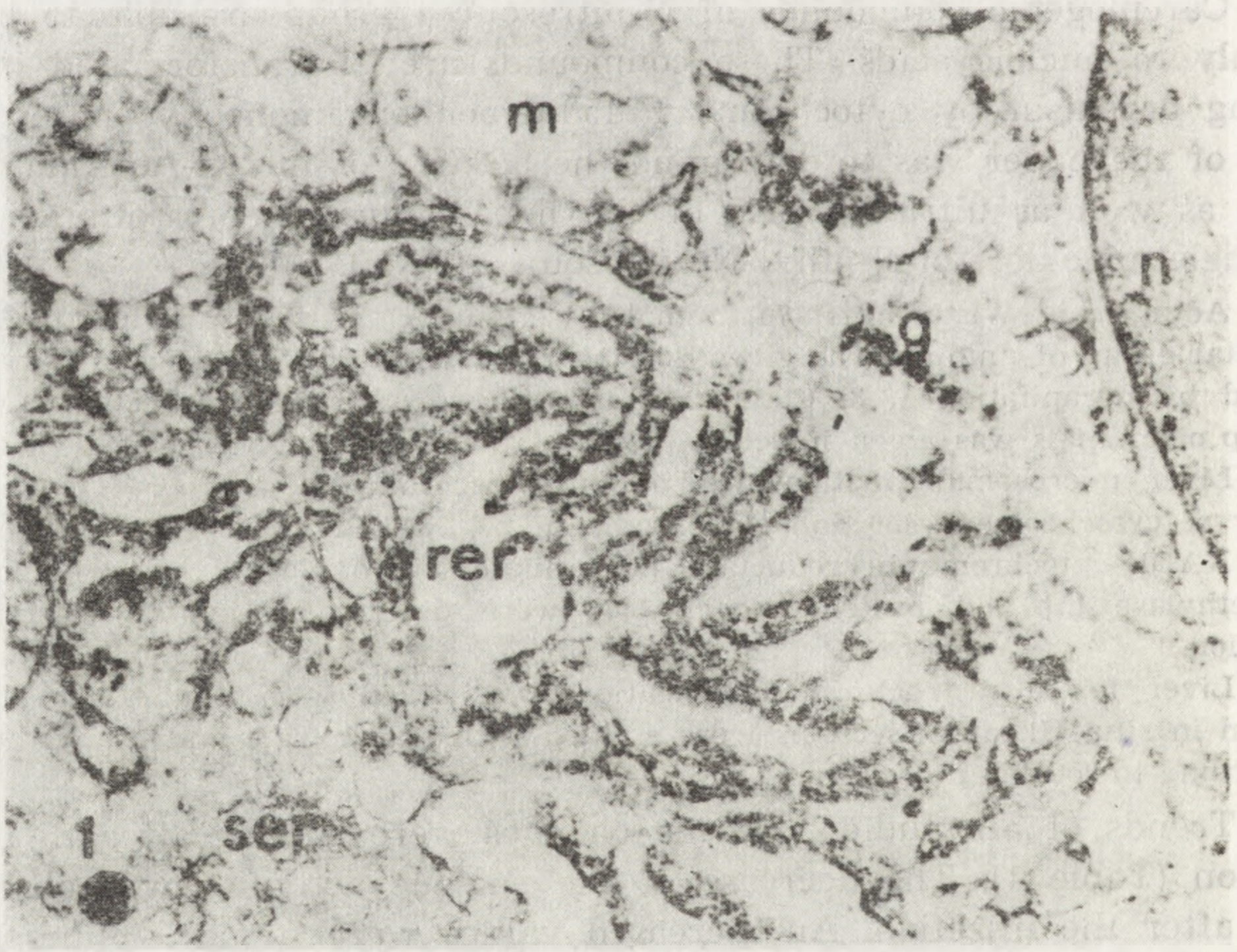

Fig. 1. Experimental group, 24 hrs after ENU-injection. Part of the hepatocyte — the 3rd zone of acinus: nucleus (n), swollen damaged mitochondrium (m), swollen short rough reticulum (rer). glycogen (g), smooth reticulum (ser). Mag. 16 300 ×

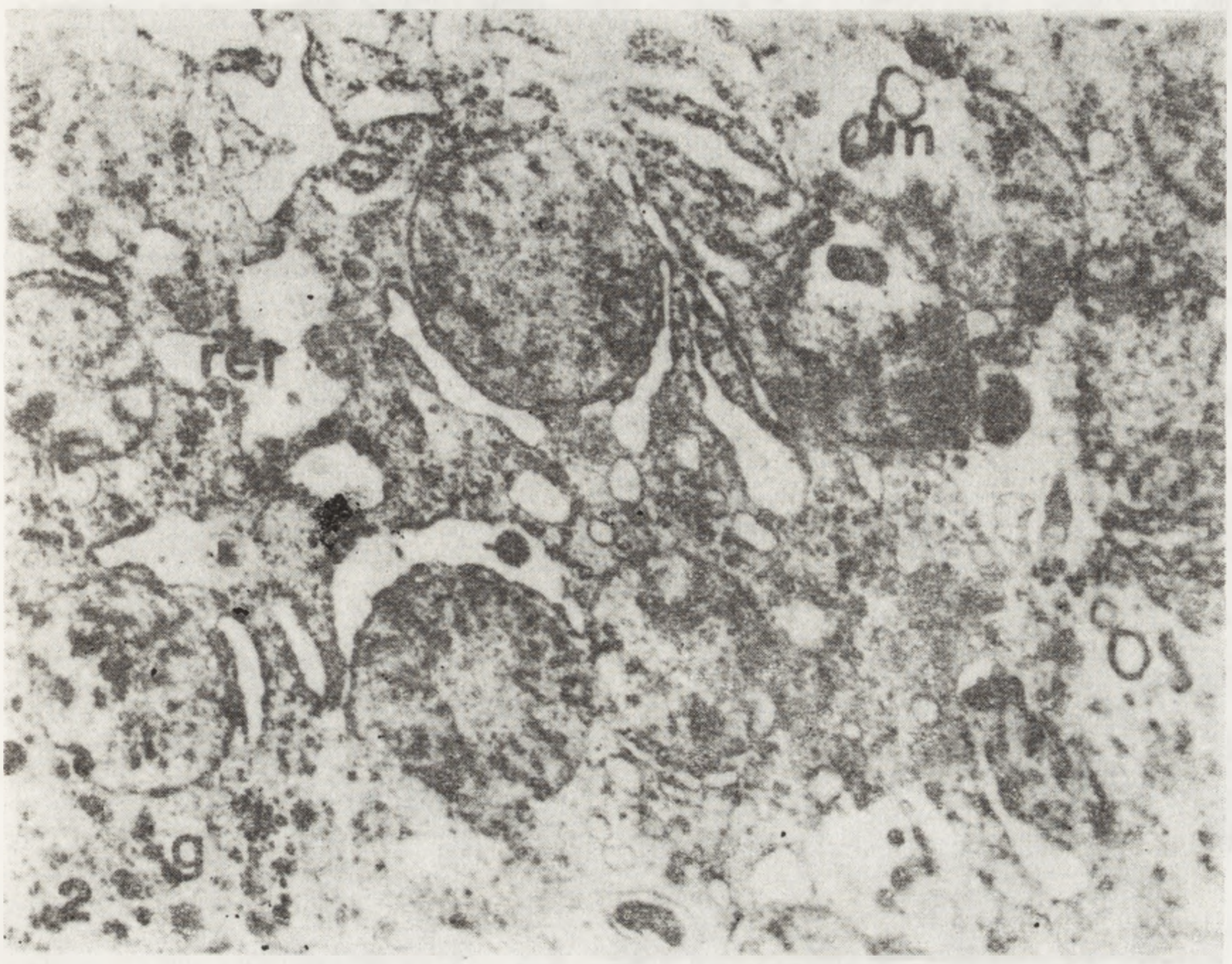

Fig. 2. Experimental group — 120 hrs after ENU injection. Part of cytoplasm of the hepatocyte 3rd zone of acinus: wide cisterns of the rough reticulum with fibrous content (rer), mitochondria with myelin figures (fm), glycogen (g). Mag. 16 300 ×

Ultrastructural investigations showed ENU-induced changes in the 3rd zone of acinus of hepatocytes: shortage of rough reticulum cisterns (Figs. 1 and 2) and wide lumenal space, filled with electron scattering substances. An increase of the smooth reticulum surface was observed in sections obtained from animals up to 24 hrs after ENU treatment (Fig. 1). Disappearence of glycogen rosettes was found in the same time (Fig. 2). Increased amount of primary and secondary lysosomes and mitochondrial injures (swelling, disappearance of continuity of lamellae and development of myelin figures) was observed in animals 6 hrs after ENU injection (Figs. 1 and 2).

It was found that single ENU injection diminished mixed-function oxidases for a short period, and than stimulated the enzyme activities. The highest enzyme activities were observed 6 hrs after ENU treatment. Ultrastructural changes were found in the 3rd zone of acini, and included qualitative and quantitative changes of endoplasmic reticulum, mitochondria and lysosomes. Ultrastructural changes were more stable than the enzymatic ones.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (495—496)

ULTRASTRUCTURAL INVESTIGATIONS OF PINEALOCYTE MITOCHONDRIA

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The aim of the studies was to investigate diurnal variations in the pinealocyte ultrastructure. Special attention was paid to the correlation between biochemical exponents of the pineal gland function and configuration of pinealocyte mitochondria in diurnal rhythm.

Investigations were carried out on 20 male Wistar rats aged 6 months. The animals were divided into two groups of 10 rats each. The first group was synchronized in LD 12 : 12, the other one was under conditions of constant darkness (D 24). After two weeks the pineal glands were excised at 11 a.m. (maximum serotonin content) and at 11 p.m. (minimum serotonin content). Then the pineal glands were fixed in 5% glutaraldehyde, postfixed in 1% OsO_4 and embedded in Araldite. Ultrathin sections were examined with a Philips EM 300 electron microscope.

In both groups mitochondria were found to be abundant within the pinealocytes at 11 a.m. and 11 p.m. These organellae were classified among various groups according to accepted criteria of mitochondrial classification [2–4]. They were found to occur in three different (metabolic) configurational states: condensed state — I and two transient states — II and III. Pinealocyte groups with mitochondria in the same configurational states were observed most frequently. They formed islets of several dozen cells. Assuming that one cell corresponds with one metabolic state, relative numbers of the pinealocytes with mitochondria in various configurational states were determined. The results are presented in Table 1 and 2.

Under conditions of LD 12 : 12 the numbers of the pinealocytes with mitochondria in the configurational state III were higher, while those in the configurational state II were lower at 11 p.m. than at 11 a.m. The inverse relations were found under conditions of D 24. These

TABLE 1

Number of pinealocytes with mitochondria in different states of configuration

States	11 a.m.	11 p.m.
I	268 (20.0%)	325 (25.7%)*
II	636 (47.3%)	459 (36.3%)*
III	439 (32.7%)	481 (38.0%)*
Total	1343 (100%)	1265 (100%)

* Statistical significant.

TABLE 2

Number of pinealocytes with mitochondria in different states of configuration

States	11 a.m.	11 p.m.
I	156 (15.2%)	330 (32.9%)*
II	356 (34.6%)	406 (40.3%)*
III	516 (50.2%)	270 (26.8%)*
Total	1028 (100%)	1006 (100%)

* Statistical significant.

findings indicate that there is no correlation between serotonin biosynthesis and pinealocyte bioenergetics. In spite of unaltered serotonin contents in LD 12 : 12 and D 24 [5], relative numbers of cells with mitochondria in various configurational states are variable. However, the results obtained testify to the correlation between pinealocyte bioenergetics and melatonin synthesis, of which maximum content is found at 24^{00}, while minimum content — at 6 p.m. under conditions of LD 12 : 12 [1]. On the other hand, in D 24 melatonin content is nearly constant throughout a day with its slight decrease at 24^{00} [1].

The occurrence of the pinealocyte groups in the form of islets in different mitochondrial configurational states suggests functional zones within the pineal gland. Thus, synchronization of the pineal gland in total, indicated by biochemical exponents of its activity, is the resultant of the function of individual pinealocytes working asynchronously.

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POSTĘPY BIOLOGII KOMÓRKI THM 11, NR 3—4, 1984 (497—500)

ULTRASTRUCTURE OF TRANSCRIPTIONALLY ACTIVE CHROMATIN AFTER HYPERTHERMIC SHOCK

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The aim of the studies was to evaluate the effect of hyperthermic shock on nuclear RNP, especially on transcription. Particular attention has been paid on preribosomal RNA synthesis under conditions of hyperthermic shock.

Hepatocytes were isolated from the livers of three-month old Wistar rats by the method of Puvion et al. [3]. The cells had formed a contiguous monolayer before they were heated at 43°C for 1 h. After heat treatment the medium was changed to MEM medium supplemented by 10 per cent calf serum. Both heat-treated and untreated cultures were subjected to spreading procedures according to Miller and Beatty [2] modified by Puvion-Dutilleul et al. [4]. For autoradiography labelling with 100 μCi/ml 5-^{3}H-uridine (sp. ac. 30 Ci/mmol C.E.A., France) was carried out according to Bouteille [1].

The most specific changes within nucleoli were observed immediately after hyperthermic shock. The granular components disappeared from nucleoli, which was accompanied by a decrease of intranucleolar chromatin strands. Autoradiographic investigations of the labelled cells exposed to high temperature revealed that immediately after hyperthermic shock there were present silver granules in fibrillar components of the as well as in condensed chromatin. After nine-hour incubation of the cells in the medium with cold uridine at 37°C all trited uridine was found within the nucleus (Fig. 1), while in the control cultures were distributed throughout the whole cell. After spreading procedures single transcriptional complexes were visible among large numbers of DNP fibrils with nucleosomal structures. Their ultrastructural appearance allowed to classify them among well-known Christmas tree figures (Fig. 2).

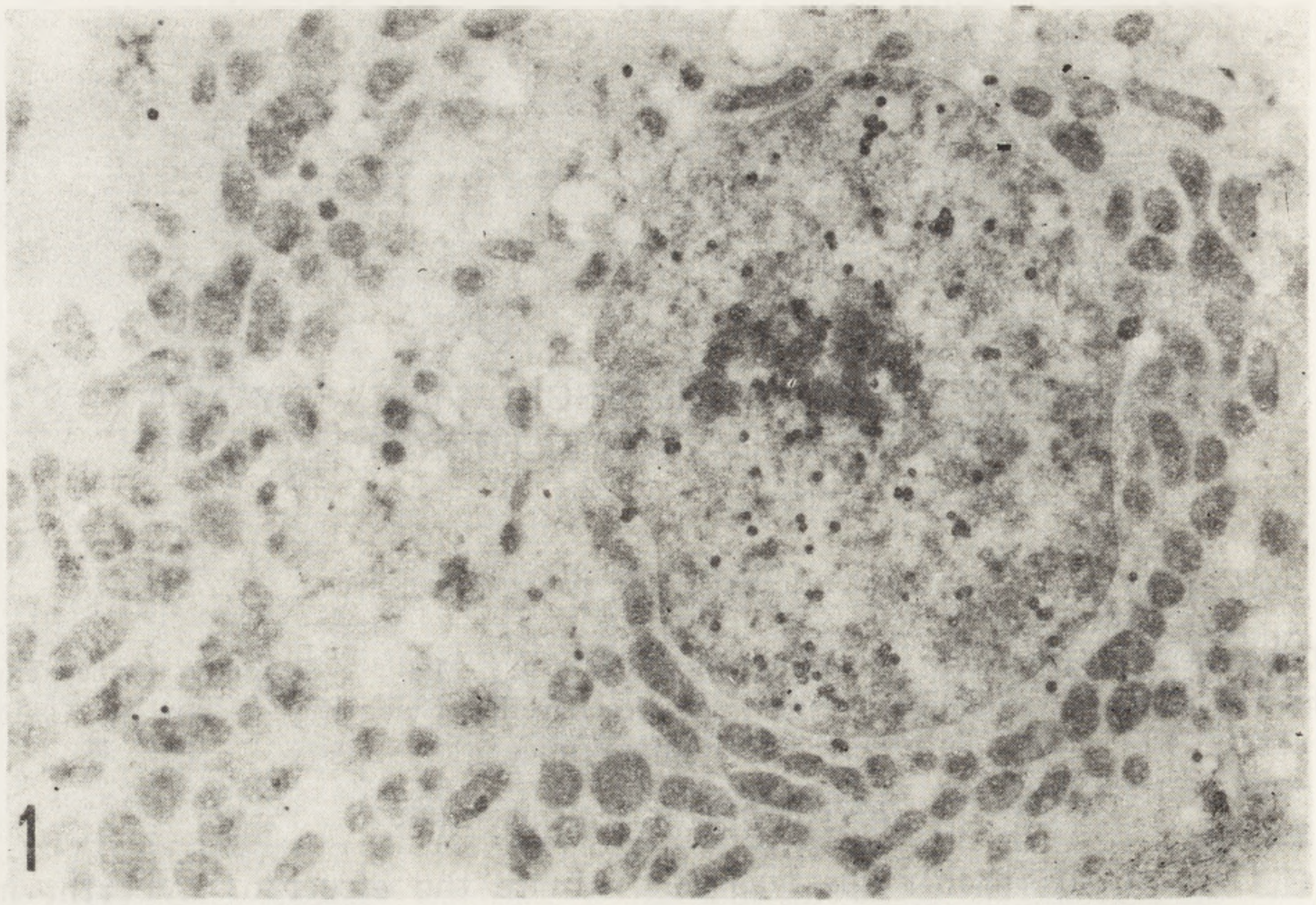

Fig. 1

Fig. 2

Our authoradiographic observations as well as the finding of nucleolar transcriptional complexes within the cells subjected to hyperthermic shock after spreading procedure, indicate that hyperthermic shock blocks preribosomal RNA synthesis and the block includes particularly posttranscriptional events.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (501—504)

STEREOLOGICAL INVESTIGATION ON MITOCHONDRIA OF MOUSE ACTIVATED BLASTOCYST

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The aim of this study was the observation of ultrastructural changes of mitochondria in mouse blastocyst kept in an experimental delay of implantation To and compared with activated blastocyst T4. Analysis of mitochondrial configurations was made using ultrastructural and morphometric methods. The study has been carried out on NMRJ mice, weighed 20–50 g groups To and T4. In every group 10 blastocysts from two animals were examined. Morphometric analysis was applied using the methods described by Weibel et al. [6]. An external compartment was omitted in the stereological study because it makes a very narrow space. In order to study the relationship between the volume of matrix, internal compartment and cristal membrane, we have introduced the following partition coefficients (E):

$$E_{ic} = \frac{V_{ic}}{S_{imm}}; \qquad E_{cav} = \frac{V_{cav}}{S_{imm}}$$

which express the volume of matrix (E_{ic}) and intracristal compartment (E_{cav}) respectively per unit of surface area of inner mitochondrial membrane (S_{imm}) [1]. Since the volume of compartments was determined for each mitochondrial profile, and surface area of the inner membrane was taken as the mean value for number of mitochondria on the micrograph, this parameter (partition coefficient) characterized nearly every mitochondrion separately. All calculations and statistical analysis were determined with T-Student and Mann-Whithney tests and were performed on a Mera 400 computer. Mouse blastocysts kept in an experimental delay of implantation. To and stimulated by estrogen injection

after 4 hours (T4) were investigated. The ultrastructural visual evaluation of mitochondria of both groups did not show any difference between them. In both experimental groups the characteristic cavities in intracristal space were observed. The group T4 volume density in inner compartment was increased significantly from 44.1% to 49.95%. On the outer hard surface the density of inner membrane (V_{im}) decreased from

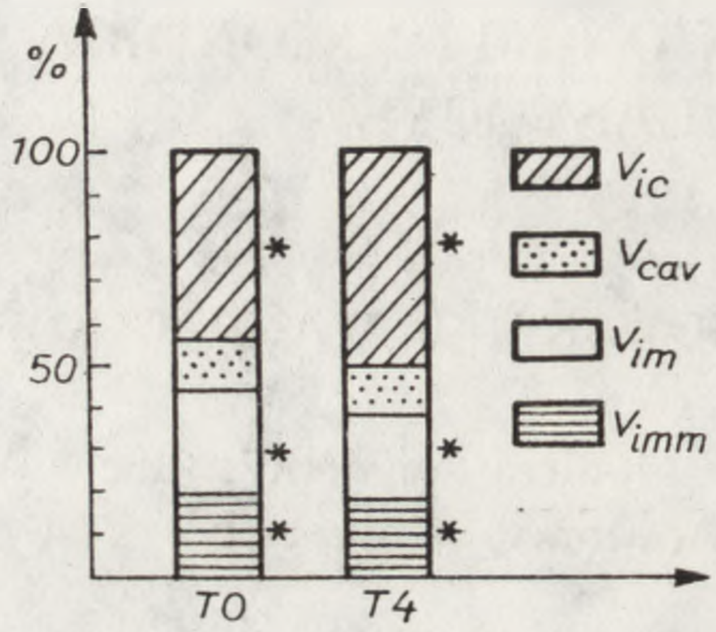

Fig. 1. Volume density of mitochondrial membranes and compartments

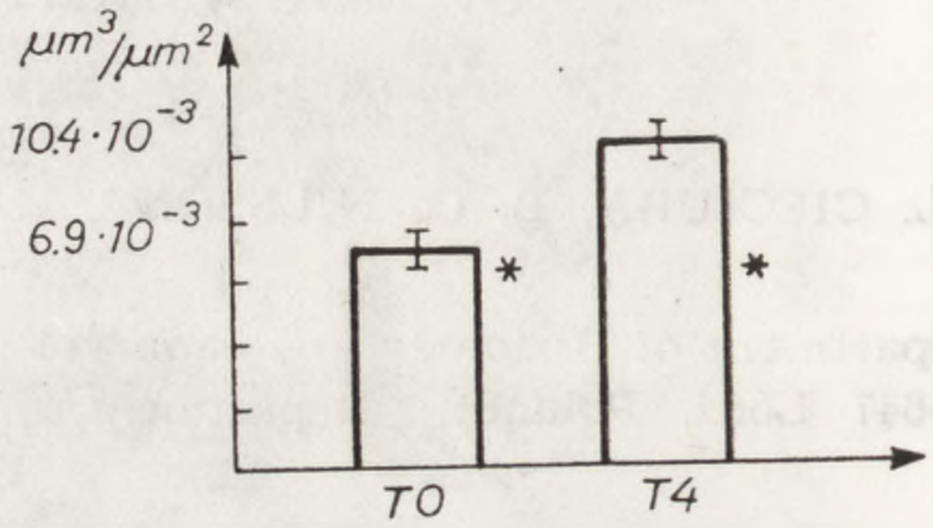

Fig. 2. Partition coefficient of inner compartment

78.93 to 59.16 and the volume density of inner membrane from 23.98 to 19.60. A decrease of volume density of the inner, most membrane was also observed from 21.29 to 18.17 (Fig. 1). Values of other parameters: volume density of intracristal compartment (V_{cav}), and volume density of the innermost membrane (V_{imm}) did not reveal the statistically significant difference. Stereological analysis of partition coefficient of matrix (E_{ic}) (Fig. 2). The results presented in the paper based upon visual evaluation and stereological analysis revealed that mitochondria of blastocysts in both experimental groups were in two different transitional configuration states. Statistical analysis of volume density of matrix and volume density of inner membrane have shown that balstocast in an experimental delay of implantation To tends to a condensed state. On the other hand mitochondria of activated blastocyst T4 transfigurates to orthodox steady state. Nilsson and Magnusson [5] reported that oxygen consumption in mouse blastocysts kept in an experimental delay of implantation $=0.25 \pm 0.01$ ml/h and in activated blastocyst 16–18 h after estrogen stimulation $= 0.5$ ml/h. The results presented in this paper are consistent with suggestion that blastocysts after estrogen stimulation reveal higher capacity to oxidative fosforylation what can be readily demonstrated as transformation of mitochondria from condensed state to orthodox one. This process is the first step in initiation of blastocyst to implantation in uterus.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (505—508)

THE EFFECT OF $Pb(NO_3)_2$ ON THE POPLAR TISSUE CULTURE AND THE ULTRASTRUCTURAL LOCALIZATION OF LEAD IN THE CULTURE CELLS

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In many research centres intensive efforts tend towards indicating correlations between the degree of environment pollution by various toxic substances and the changes in plant growth development. The subject of such research are also changes caused by lead. The present study was undertaken to establish the influence of lead nitrate on morphology of the tissue culture (particularly the changes in the growth and the anthocyanin accumulation). Localization of the lead in the cells of poplar tissue culture was also done by means of a transmission electron microscopy.

The conditions of the callus production and the poplar tissue culture was based on the data published by Walter [3], Venverloo [2] and Chalupa [1]. Fragments of two-week-old cultures were (in sterile conditions) exposed to the 24-hour-long incubation in the water solution of $Pb(NO_3)_2$ at the concentration 300, 600, and 1800 ppm of Pb. The control was the material incubated in distilled water. The incubation was carried out in darkness, at the temperature 20°C. Then the fragments were placed in the medium to the 25 ml Erlenmayer flasks. The cultures were kept for two weeks in darkness or in the light with the intensity of about 9 $W \cdot m^{-2}$. The material to study in a transmission electron microscopy was prepared according to standard method. The sections were examined under a JEM 7A transmission electron microscopy, unstained or after poststaining in uranyl acetate and lead citrate.

After 24 h from the moment of exposure of the tissue culture to light in the control cultures as well as in the cultures exposed to the action of the plumbous nitrate at the lowest concentration used, i.e.

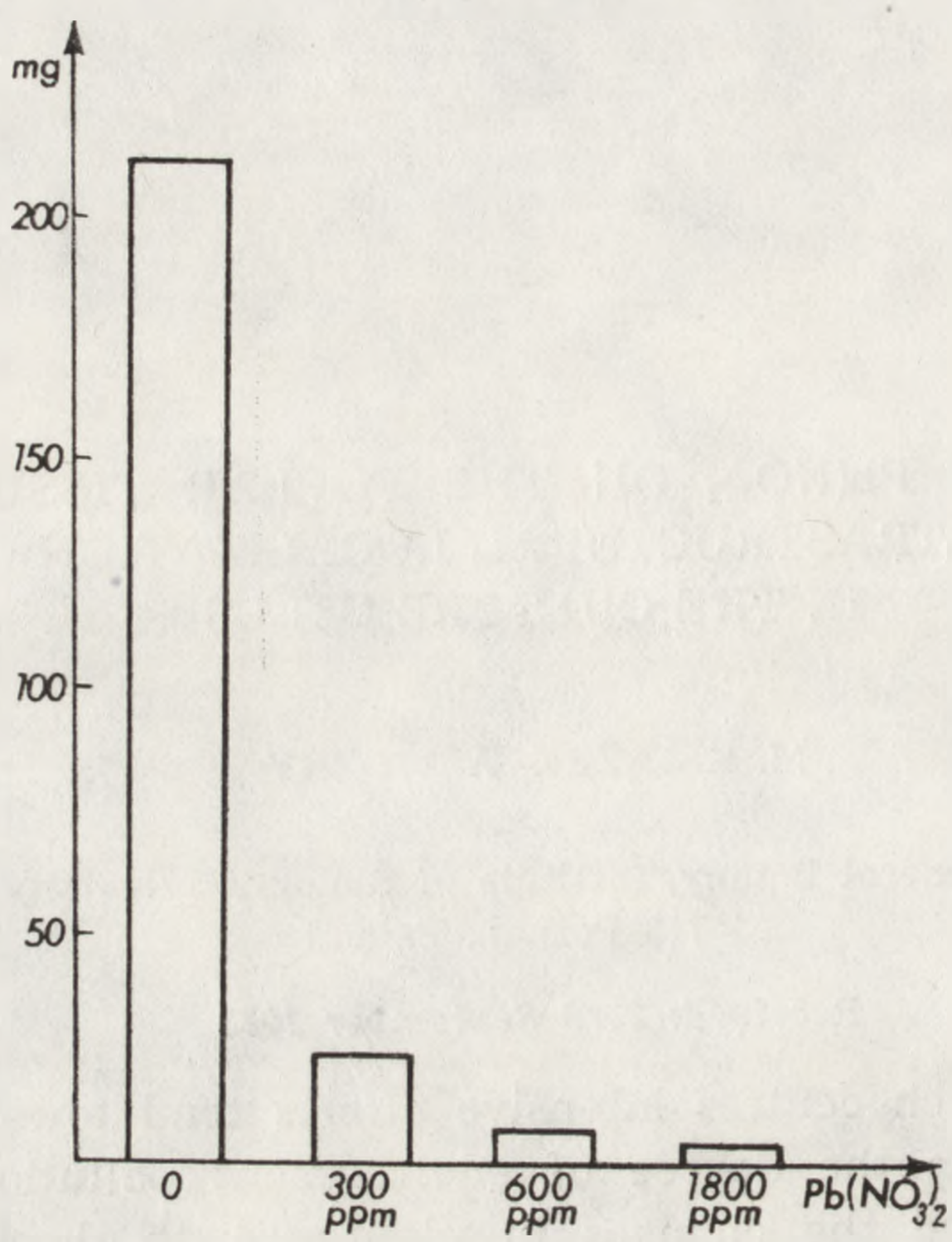
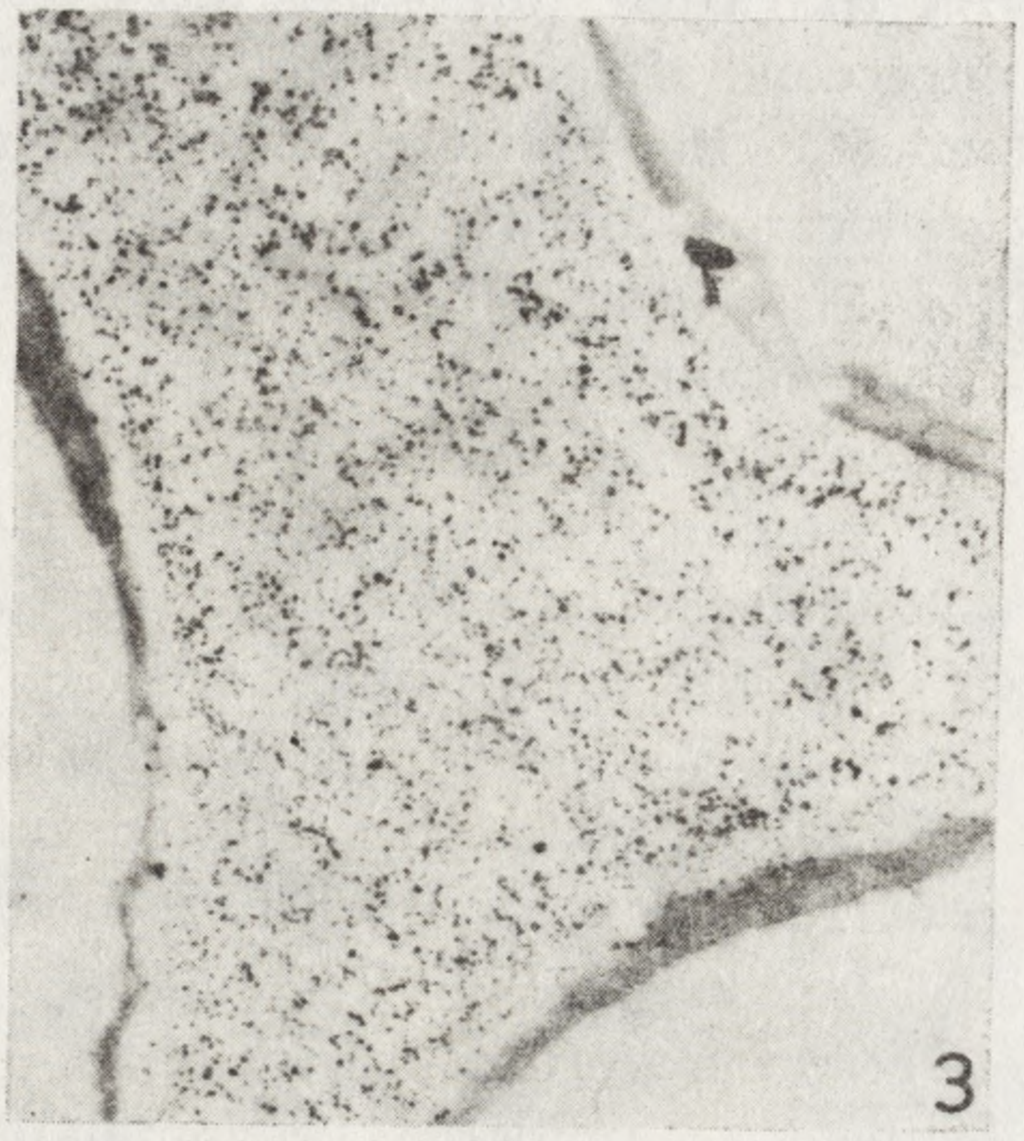
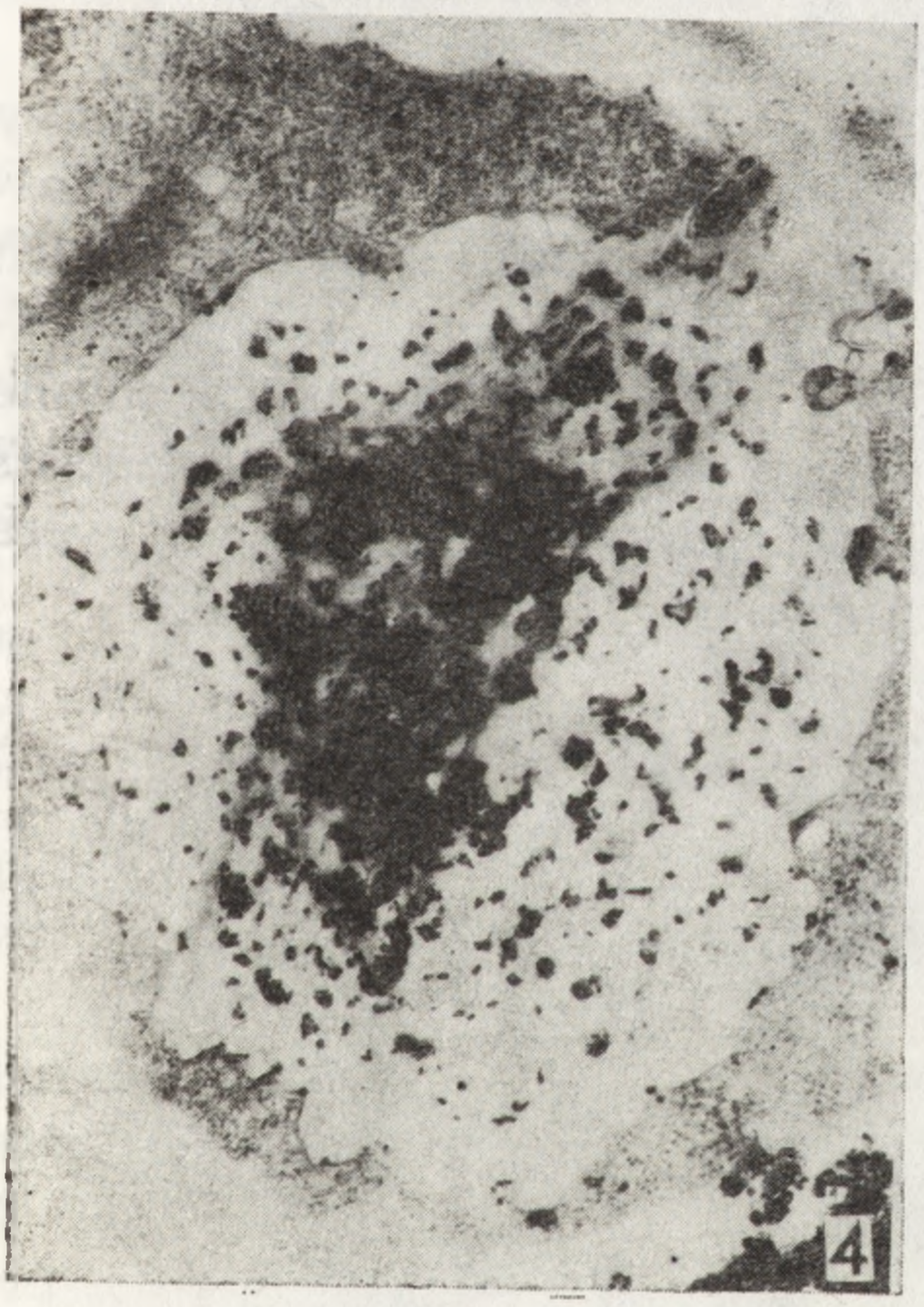


Fig. 1. The fresh weight of the tissue culture after 24 h of incubation in $Pb(NO_3)_2$ and then a two-week cultivation on a medium without $Pb(NO_3)_2$. Mean value of 5 measurements

300 ppm of Pb, the accumulation of the anthocyanin were observed. In the tissue culture exposed to the $Pb(NO_3)_2$ at the highest concentration (1800 ppm of Pb) the anthocyanin synthesis was not found even after a two-week exposure to light. In the conducted experiments a considerable difference in the tissue culture growth was observed. The cultures incubated in the distilled water were (after their transfer to the medium) characterized by almost ten times higher increase of fresh weight than the cultures incubated in the solution of $Pb(NO_3)_2$ at the concentration 300 ppm of Pb. The cultures incubated in the solutions at the concentrations of 600 and 1800 ppm of Pb showed only small increase of fresh weight (Fig. 1). Such a considerable difference in fresh weight increase in response to lead contamination resulted probably from the disturbance of cell division. The justification of this statement are other authors' results.

The main components of observed by us control tissue culture, were differentiated in size, strongly vacuolated cells of nearly oval shape. Numerous pictures of the cells being at different stages of cytokinesis show a considerable division activity of the culture cells.





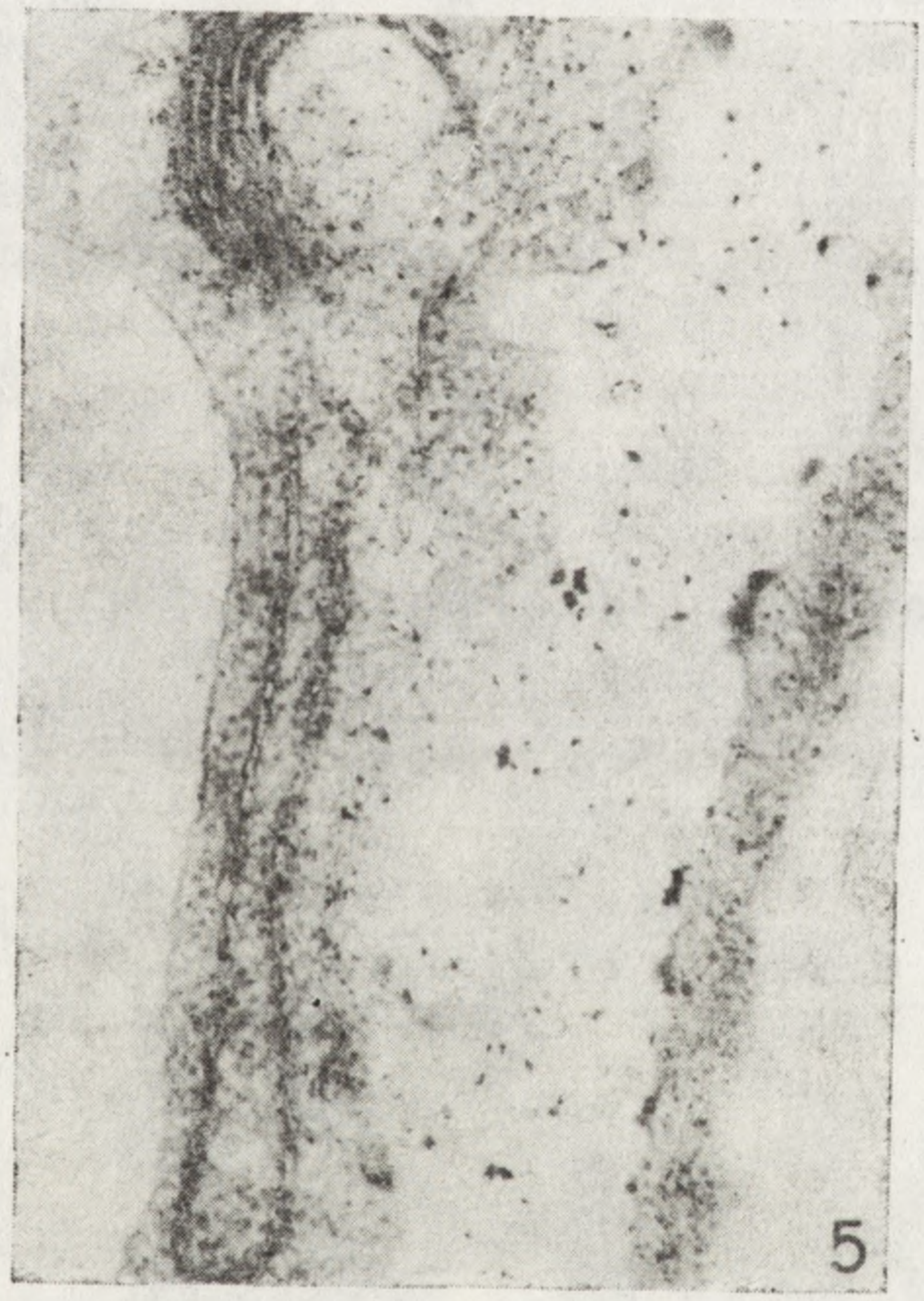


Fig. 2–5. Fragments of two-week-old dark tissue culture after 24 h of incubation in $Pb(NO_3)_2$. Fig. 2. Fragment of the cells from a surface layer of the culture (1800 ppm Pb) × 25 000; Fig. 3. Pb deposits in the intercellular space (1800 ppm Pb) × 3000; Fig. 4. Paramural body with Pb deposits (600 ppm Pb) × 13 000; Fig. 5. Pb deposits in endoplasmic reticulum, dictyosome and cell wall (1800 ppm Pb) × 20 000

At the same time, the ultrastructural research of poplar tissue culture cells affected by the Pb salt was made. The tissue culture cells affected by the $Pb(NO_3)_2$ at a concentration of 300 ppm of Pb had well preserved protoplasts. In spite of the fact that some cell organelles contained Pb deposits, no changes were observed in their appearance in comparison with the cells of control tissue culture. On the other hand, the tissue culture affected by $Pb(NO_3)_2$ at a higher concentration looked different. Among the cells of the cultures incubated in the water solutions of plumbous nitrate at a concentration of 600 ppm of Pb, there existed both cells which had undisturbed protoplasts and cells with a strongly degenerated protoplasts as well. That phenomen increased in the cells which were affected by solutions of plumbous nitrate at a concentration of 1800 ppm of Pb. The protoplasts of most cells which were directly exposed to $Pb(NO_3)_2$ at that concentration, were degenerated and their remains were dense for the electrons (Fig. 2). In the tissue culture cells, lead accumulated predominantly in the intercellular spaces and in the cells walls (Figs. 3 and 5).

In the cells of tissue cultures directly exposed to $Pb(NO_3)_2$ heavy deposits were also observed in spaces between the cell walls and plasmalemma, and sometimes in paramural bodies of different size which were probably created by formation of lead-containing pinocytic vesicles (Fig. 4). Apart from the major sites of lead deposition, i.e. in the cell walls and intercellular spaces, the metal in form of a small deposits was also observed almost in all cell organelles. However, they were numerous in the ER and dictyosomes (Fig. 5).

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (509—512)

ULTRASTRUCTURAL LOCATION OF LEAD IN THE CELL WALLS OF *ALLIUM CEPA* L. ROOTS

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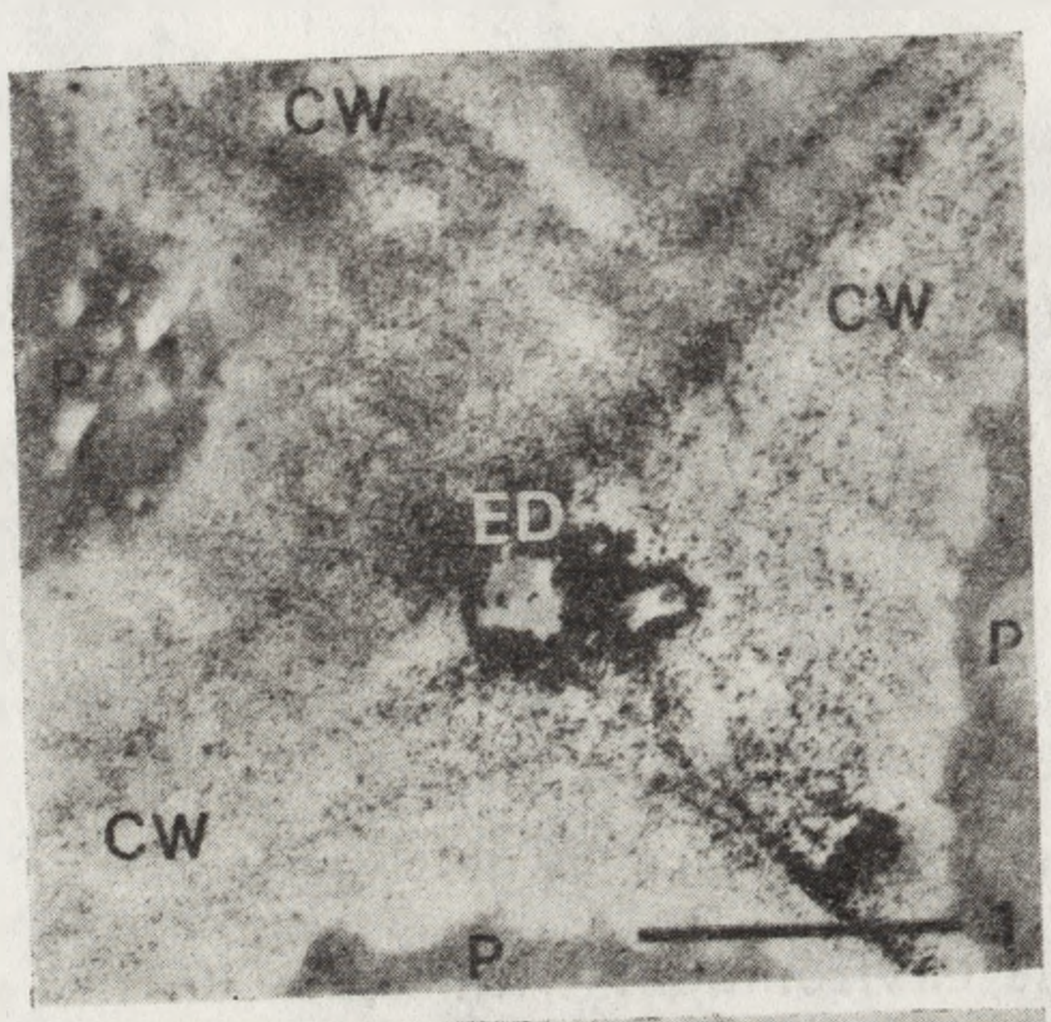
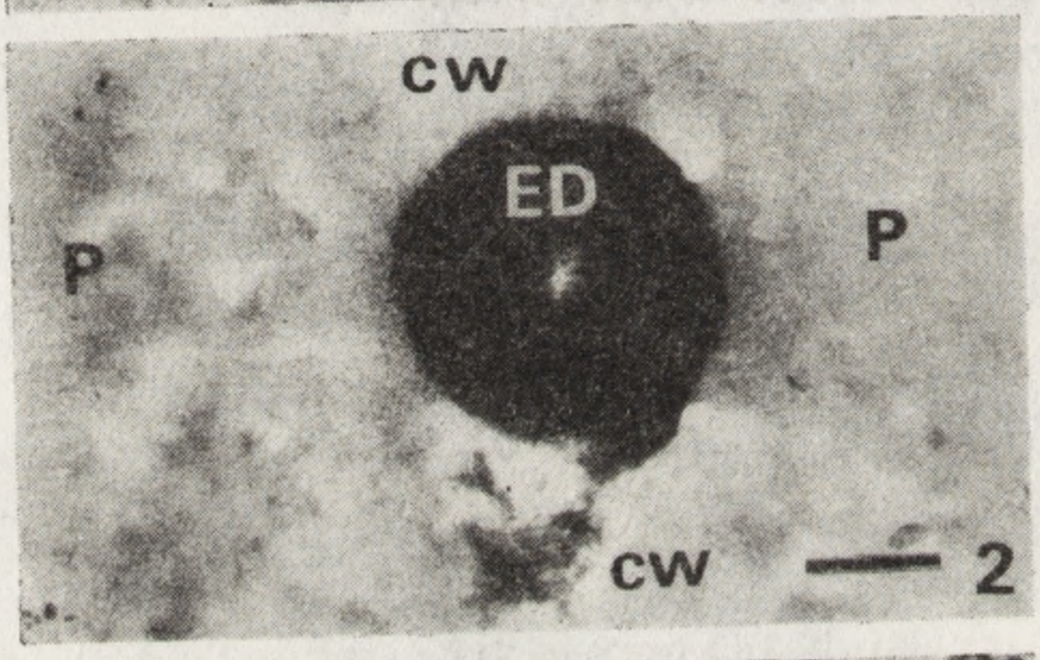
The contamination of the environment with heavy metals continues to be actual. Plant organisms exhibit a much higher tolerance to poisoning with these substances than do animal organisms. This increases the danger to humans and animals since large quantities of heavy metals (e.g. lead) can be included into the food chain. The question arises what is the cause of such efficient lead metabolism in plants and what is lead localization.

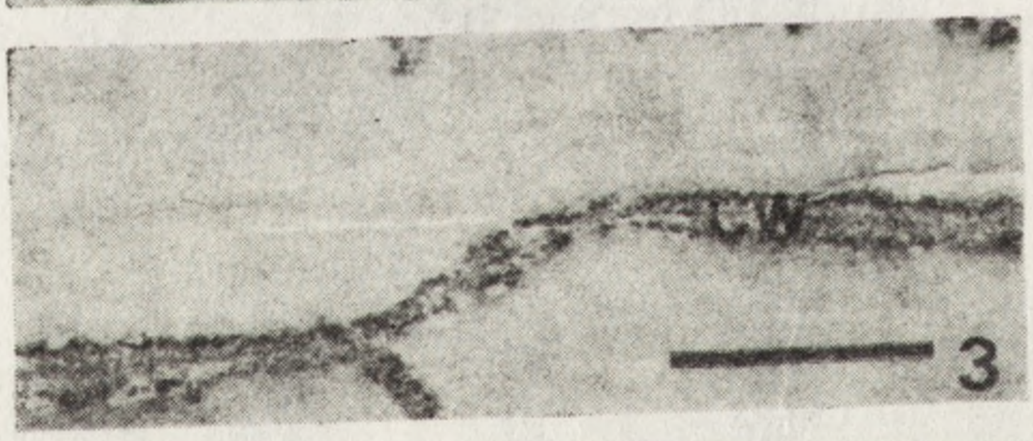
Experiments were carried out on adventitious roots of onion (*Allium cepa* L.). Pb^{++} ions were supplied to the roots of the plants in a nonlethal dose of 2.5 ppm Pb^{++} dissolved in water and 15 ppm Pb^{++} added to an incomplete Hoagland medium (without PO_4^{--} ions) in the form of lead chloride or nitrate for 5 min to 24 h. The root apical meristems were further investigated by routine electron microscopy techniques. The roots were fixed in glutaraldehyde and osmium tetroxide.

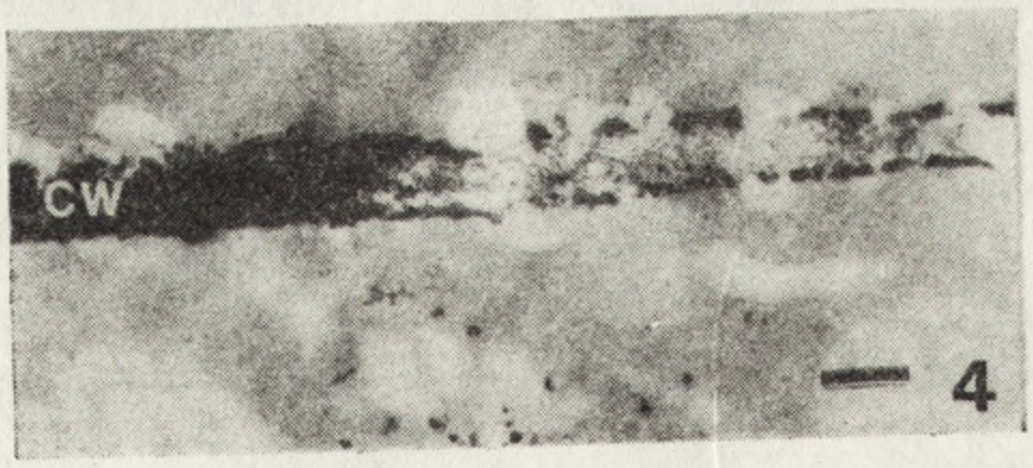
The observations demonstrated that lead accumulates first on the root surface (5 min) and then penetrates in radial direction through the middle lamella of the covering tissue (root cap) cells. Beginning with 60 min of incubation fine-grained lead deposits were observed in the middle lamellae and intercellular spaces throughout the proparenchyma. With prolongation of incubation time the amount of lead increased until finally (after 24 h of incubation) lead granules accumulated around the center and crystalloid of lead compounds was formed (Figs. 1 and 2).

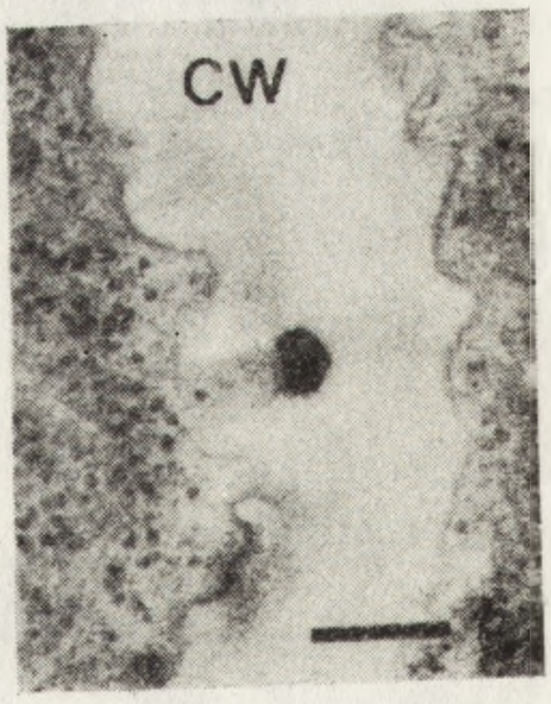
In the initial incubation period lead penetrated also to the cell protoplast, as indicated by the appearance of vacuoles containing electron-dense lead deposits (2–3 h incubation). At the same time accumulation of large amounts of lead was observed at the border between the cell wall and the plasmalemma. This process intensified as the incubation











time was prolonged (Fig. 3). The process was accompanied by an increase in the plasmalemma surface due to formation of tubular convexities over which spherical aggregations of lead deposits appeared (Figs. 5 and 6). Finally some segments of the cell wall become strongly saturated with lead (Fig. 4).

Thus, as the result of lead translocation through the root apoplast, large amounts of lead accumulated within the middle lamellae and the intercellular spaces (as the result of reaction with pectin substances), and, due to the release of lead from the protoplast into the cell wall, the cellulose part of this wall became strongly impregnated. This means that one of the main causes of the tolerance of plants to lead is their ability to inactivate large quantities of this metal in the cell walls.

In this way lead is not toxic though accumulated in plants.

Fig. 1. Fine grained lead deposits (ED) in middle lamella of cell wall (CW) of proparenchyma cells. Incubation 24 h in $PbCl_2$ with Pb^{++} concentration 15 ppm given in medium. Unstained section, P — cytoplasm, [——] 1 μm

Fig. 2. Crystalloid of lead compounds (ED) in cell wall (CW). Incubation 24 h in $Pb(NO_3)_2$ with Pb^{++} concentration 15 ppm given in medium. Unstained section, P — cytoplasm, [——] 0.1 μm

Fig. 3. Cell wall (CW) saturated with lead, at the border of plasmalemma. Incubation 6 h in $Pb(NO_3)_2$ with Pb^{++} concentration 2.5 ppm given in water. Unstained section, [——] 1 μm

Fig. 4. Cell wall (CW) saturated with lead. Incubation 24 h in $PbCl_2$ with Pb^{++} concentration 15 ppm given in water. Unstained section, [——] 0.1 μm

Fig. 5. Lead accumulation above tubular convexities of plasmalemma (marked with hyphens) within cell wall (CW). Incubation 24 h in $Pb(NO_3)_2$ with Pb^{++} concentration 15 ppm given in medium. Section stained, [——] 0.1 μm

Fig. 6. Tubular convexities of plasmalemma with accumulated lead deposits on the side of the cellular wall (CW). Incubation 24 h in $PbCl_2$ with Pb^{++} concentration 15 ppm given in medium. Section stained, [——] 0.1 μm

POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (513—516)

THE MECHANISM OF POSTPROPHASE NUCLEOLUS FORMATION

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Nucleolus is a structure that occurs periodically in the cell cycle. It is formed during telophase and disappears during late prophase. The period of nucleolus disintegration is in some plants prolonged to form persistent nucleolus called hereto postprophase nucleolus. The postprophase nucleolus is a ribonucleoprotein structure revealing physical continuity of interphase nucleolus but lacking its function and structural organization.

The experiment was made with meristematic cells of *Lupinus luteus* L. roots, a species that proved to have about 50% of metaphase cells with postprophase nucleolus.

The study were carried out in a selectively synchronized population of binucleate cells having treated the roots with 0.2% caffeine solution. The total life cycle of binucleate cells was 12 hours including 3.4 hours of mitosis. The duration of each period of interphase marked with the use of ^3H thymidin was respectively G_1 — 1.9 h; S— 5.7 h; G_2 — 1 h. During the period G_2 of the cell cycle the metabolism of nucleic acid was interrupted with the treatment of the following: 5-fluorodesoxyuridin (FUDR), 5-fluorouracil (FU), actinomycin D (Act. D), ethidium bromide (EB), gibberelic acid (GA_3).

It was found that blocking the availability of the template in the presence of actinomycin D or ethidium bromide does not influence the delay of nucleolus disapperance (Fig. 1). The formation of postprophase nucleolus is connected with the disturbance in maturation of ribosomal RNA which can be inducted experimentally by treating the cells of lupin with FUDR or FU — substances that interfere in nucleic acid metabolism on the level of nucleotide pools. The morphological result of such disturbance was the hypertrophy of nucleolus always accom-

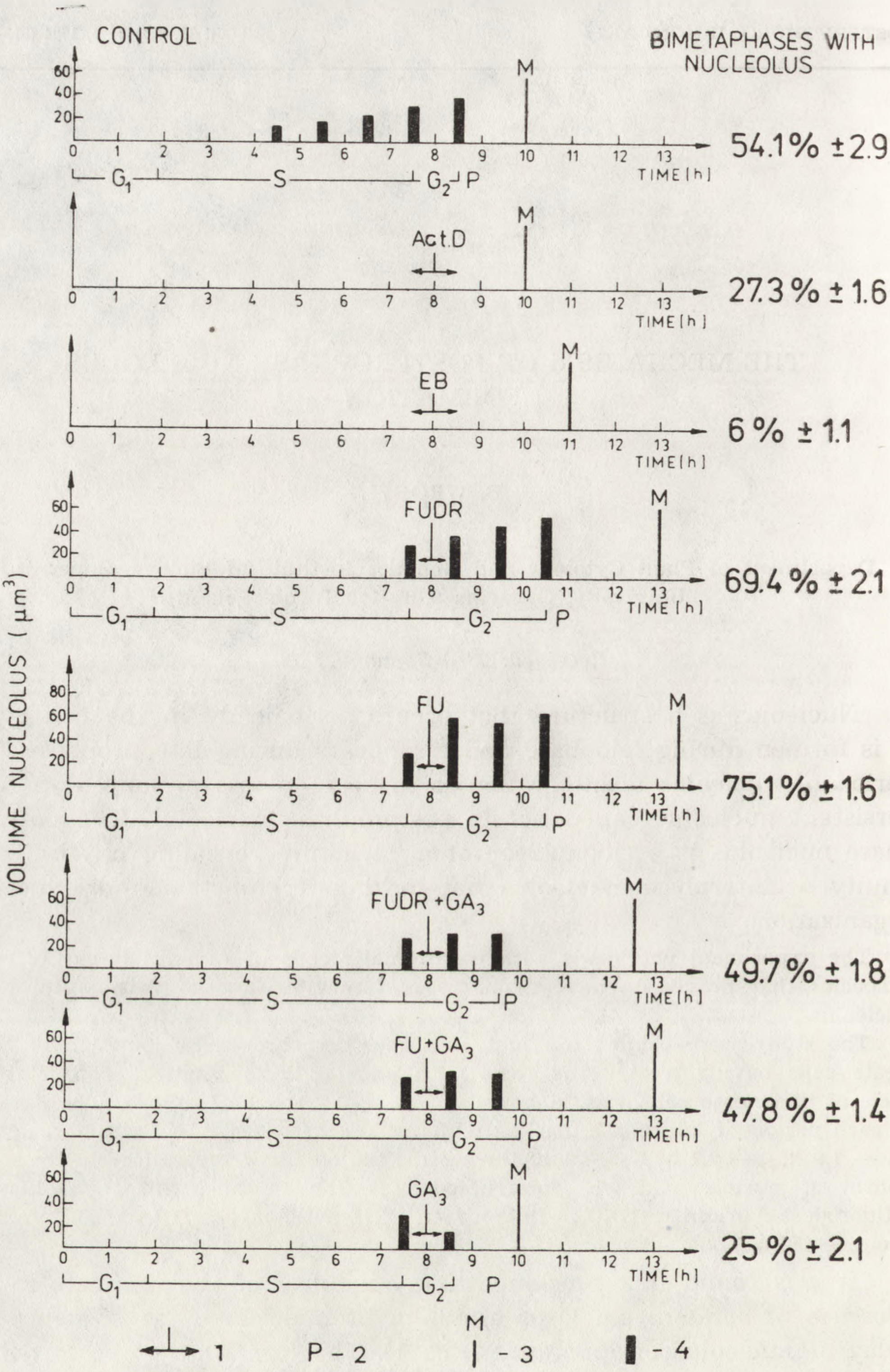

Fig. 1. Volume of nucleoli and number cells with postprophase nucleolus; *1* — one hour incubation in solution: Act. D (25 mg/l), EB (12.5 mg/l), FUDR (50 mg/l), FUDR (50 mg/l) + GA_3 (0.1 mg/l), FU (75 mg/l), FU (75 mg/l) + GA_3 (0.1 mg/l), GA_3 (0.1 mg/l); *2* — moment at which the population of binucleate cells starts prophase; *3* — moment at which maximum metaphases were noted in the population of binucleate cells; *4* — nucleolus volume

panied by the increase in the number of cells containing postprophase nucleolus.

In the mechanism of postprophase nucleolus formation is involved the plant hormone — gibberellin (GA_3) that is said to speed up the processing in rRNA. It was found that gibberellin alone reduces the number of cells containing postprophase nucleolus which is accompanied by the diminution in volume of nucleolus. Gibberelin used together with FUDR or FU suppressed the effects of these inhibitors by carrying away hypertrophy of nucleolus and decreasing the number of cells containing postprophase nucleolus.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (517—520)

ULTRASTRUCTURAL EXAMINATIONS OF REGENERATION OF EPI-THELIUM IN RATS' ILEUM AFTER ADMINISTRATION OF VIBRAMYCIN AND ENTEROHORMONES

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Our histological and morphometric examinations indicate, that vibramycin delays regeneration of intestinal epi-thelium by several and more hours and causes an increased formation of numerous mucogenic cells, the number of which greatly exceeds normal limits. The administration of pentagastrin and of glucagon accelerates regeneration of epi-thelium in animals, to which vibramycin was not administered and delays the disturbances of regeneration in vibramycin treated animals.

The ultrastructural examinations were carried out with the purpose to discover some elements responsible for disturbances of regeneration of intestinal epi-thelium, following administration of vibramycin.

The material for examinations was taken from three groups of animals: treated with vibramycin, treated with glucagon and pentagastrin of intestinal epi-thelium, following administration of vibramycin.
and of control rats. The segmental desquamation of intestinal epi-thelium was performed according to the method previously reported [1, 2]. The material was fixed in 6.5% solution of glutaraldehyde, dissolved in phosphate buffer and then in 1% solution of osmic acid; the fragments of intestine were embedded in EPON 812, according to Luft. The regeneration and differentiation of epi-thelium was examined after desquamation on all levels of crypts and villi in the following time periods: 4, 8, 12 and 24 hours.

In the bottom of crypts a slight increase of number of Paneth's cells was observed, which became distinctly oedematous, mostly because of enlargement of zymogenic granules. This phenomenon, significantly intensified in rats treated with vibramycin, was accompanied by clari-

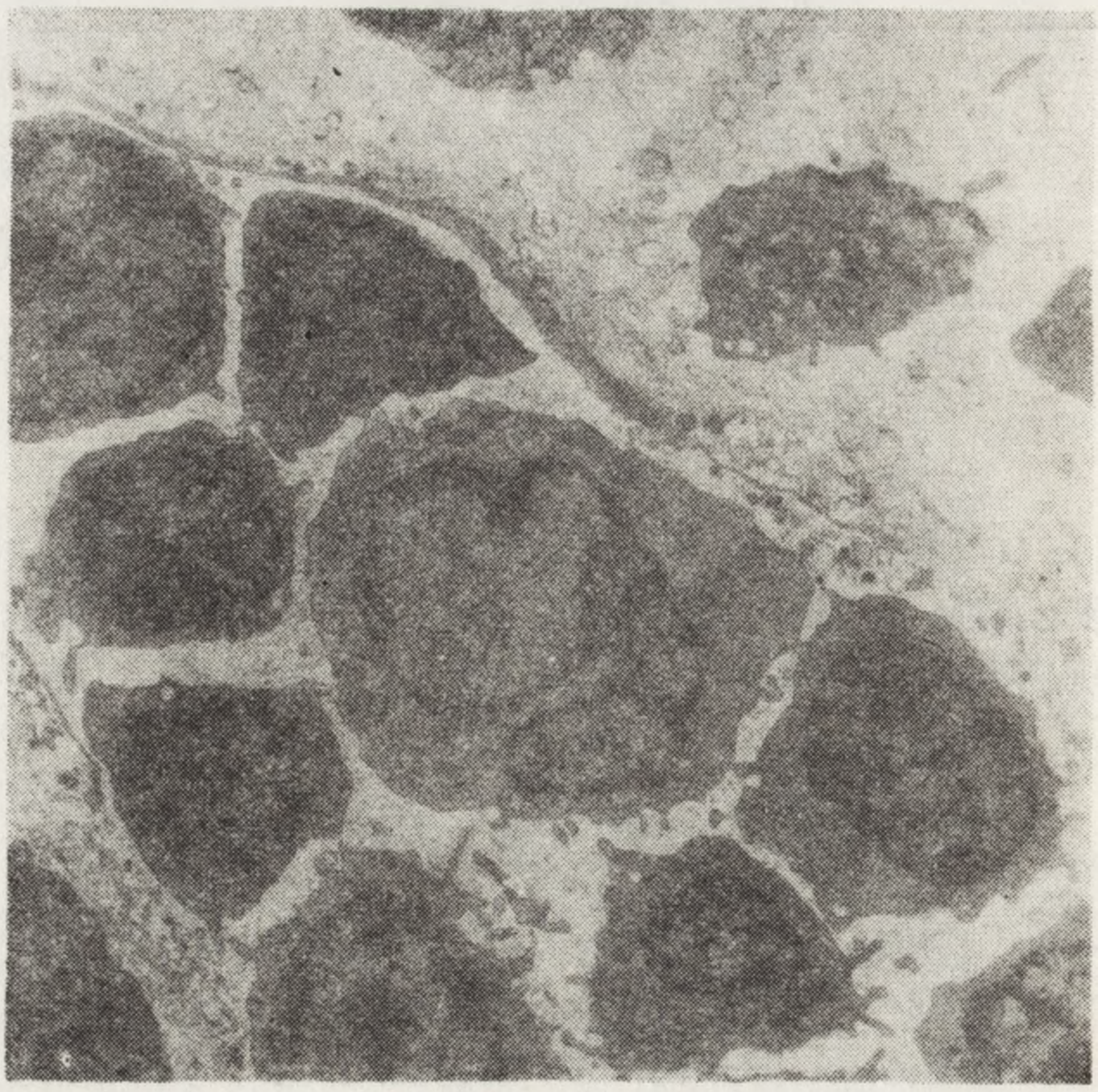

Fig. 1. Multi-layer propagation of dedifferentiated epithelial cells in renewal area of desquamated villus. Note the long processus over oedematous basal part of the villus. Vibramycin treated rat killed 8 h. after desquamation of epithelium. 6 500 ×

Fig. 2. Parts of too flattened cells in renewal area of desquamated villus in control rat killed 12 h. after desquamation of epithelium. 12 000 ×

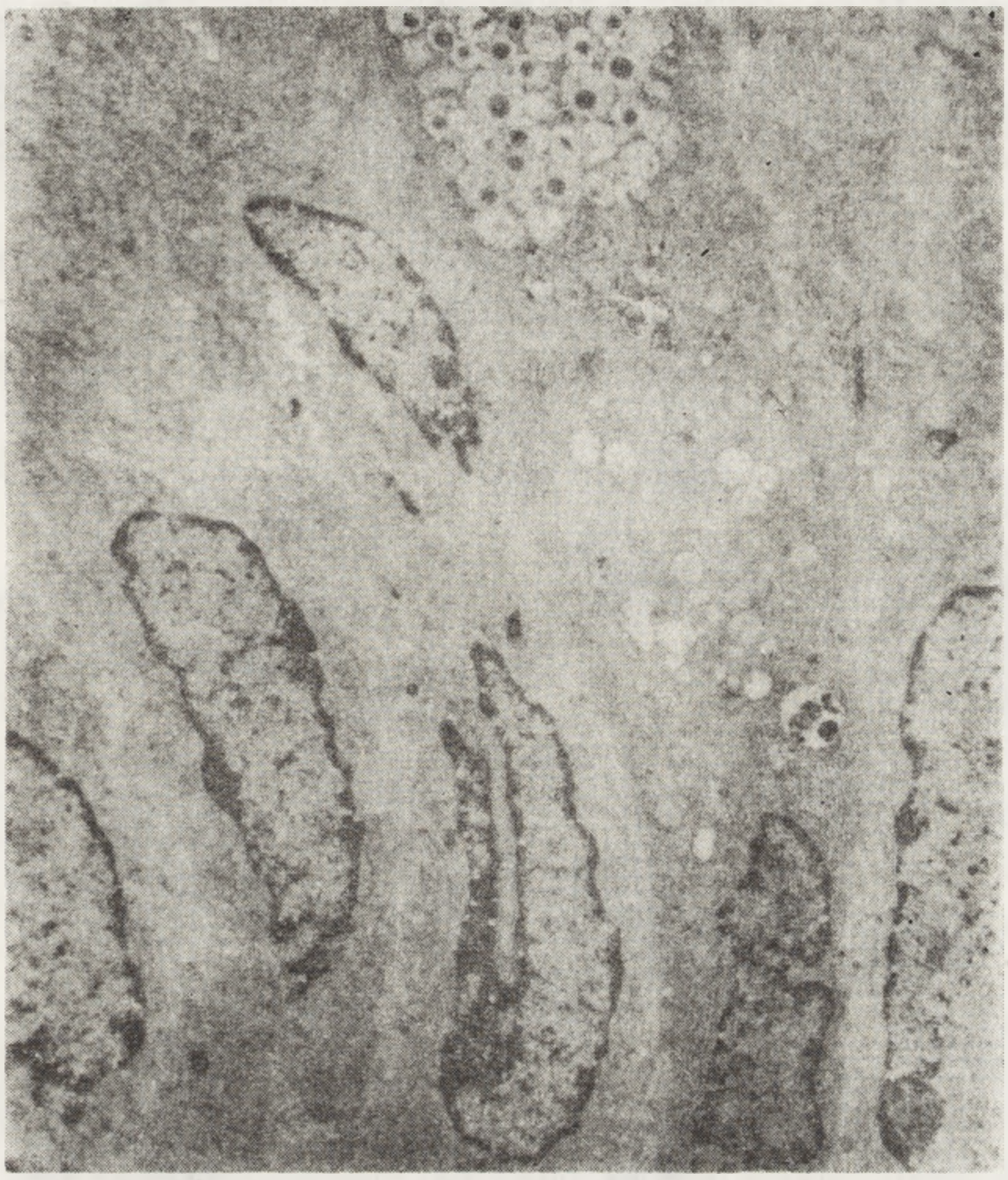

Fig. 3. Oligomucous cell (granular) between monolayer cubic cells. Lower area of differentiation zone in a control rat killed 8 h. after desquamation of epithelium. 12 000 ×

fication of zymogen granules and by changes of amorphous material of different electron density flocculent or less often microgranular one. The first signs of cells differentiation were found in those located close to Panth's cells. As its result in some cells rough endoplasmic reticulum was developed, in others numerous mitochondria were visible.

In the lower 1/3 part of crypts and in animals, to which vibramycin was given above this limit, cells containing mucous globuli were found, which however did not form calyces, typical for goblet cells. Besides them comparatively frequently the presence of cells was noted, which contained typical mucous globuli and granules filled with partially homogenous or flocculent material, having ultrastructural character of mucus. Such mucous, granular and intermediate cells were found more often in animals treated with vibramycin and their presence was observed both in crypts and on villi.

The cells of enterocytes series did not exhibit any distinct ultrastructural disturbances in their differentiation, irrespective of administration of vibramycin or hormones to animals.

In creeping area of epi-thelial cells on the villi, significant pleomorphism of undifferentiated cells was observed — some of them resembled mononuclear macrophages or histiocytes. Their axons adhered to the basal membrane and on their free surface few and irregularly located microvilli were visible. These cells possessed comparitively frequently phagosomal vesicles and mucous globuli.

In control animals a monolayer of distinctly flattened cells was formed by the creeping process. In animals treated with vibramycin and hormones several layers of young, undifferentiated cells were formed, which in process of delayed differentiation and maturation formed gradually a monolayer epithelial coat of the intestinal villi.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (521—524)

DIFFERENTIATION OF CHLOROPLASTS STRUCTURE IN BUNDLE SHEATH CELLS IN MAIZE UNDER LOW INTENSITY LIGHT OF VARIOUS WAVELENGTH

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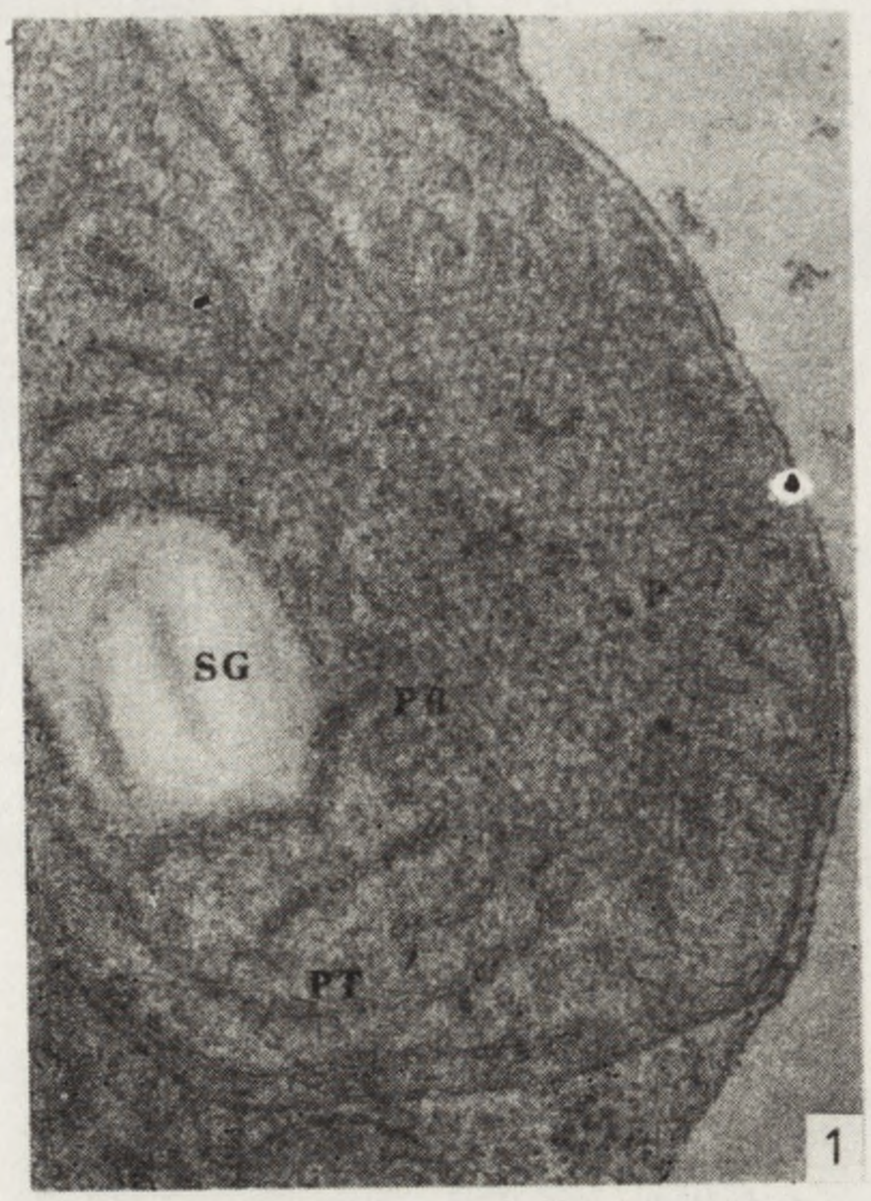
Etiolated 8-day-old maize seedlings were illuminated for 72 h by continous white, blue and red low intensity light. Photon numbers of white, blue and red light were the same. Fifth segment chloroplasts of the second leaf were analyzed.

Maize is a C_4 type plant with a significant chloroplasts dimorfism between mesophyll and bundle sheath cells. Mesophyll chloroplasts are of granal type, typical for higher plants whereas bundle sheath chloroplasts have nearly no grana (Brangeon 1973, Laetsch 1974).

Etioplasts in second leaf of the 8-day-old etiolated maize seedlings are fully developed. Usually they have one big paracrystalline prolamellar body and a small number of perforated thylakoids (Fig. 1). Numerous rybosomes and starch grains are present in stroma. Mesophyll and bundle sheath etioplasts are not dimorphic.

I. Low intensity white light induced prolamellar body transformation and later recrystallization, i.e. reformation of the paracrystalline structure. Prolamellar bodies disintegrate into few smaller ones and their dimensions gradually decrease during chloroplast development. Prolamellar body decay is accompanied by stroma thylakoids development and the grana formation. 3–4 small paracrystalline prolamellar bodies and numerous grana are present on chloroplast sections after 24 h of illumination. Longer illumination (up to 72 h) leads to formation of even higher number of small paracrystalline prolamellar bodies with numerous long grana containing usually 2–5 narrow compartments (Fig. 2).

II. Continous low intensity blue light is the most effective in chlo-



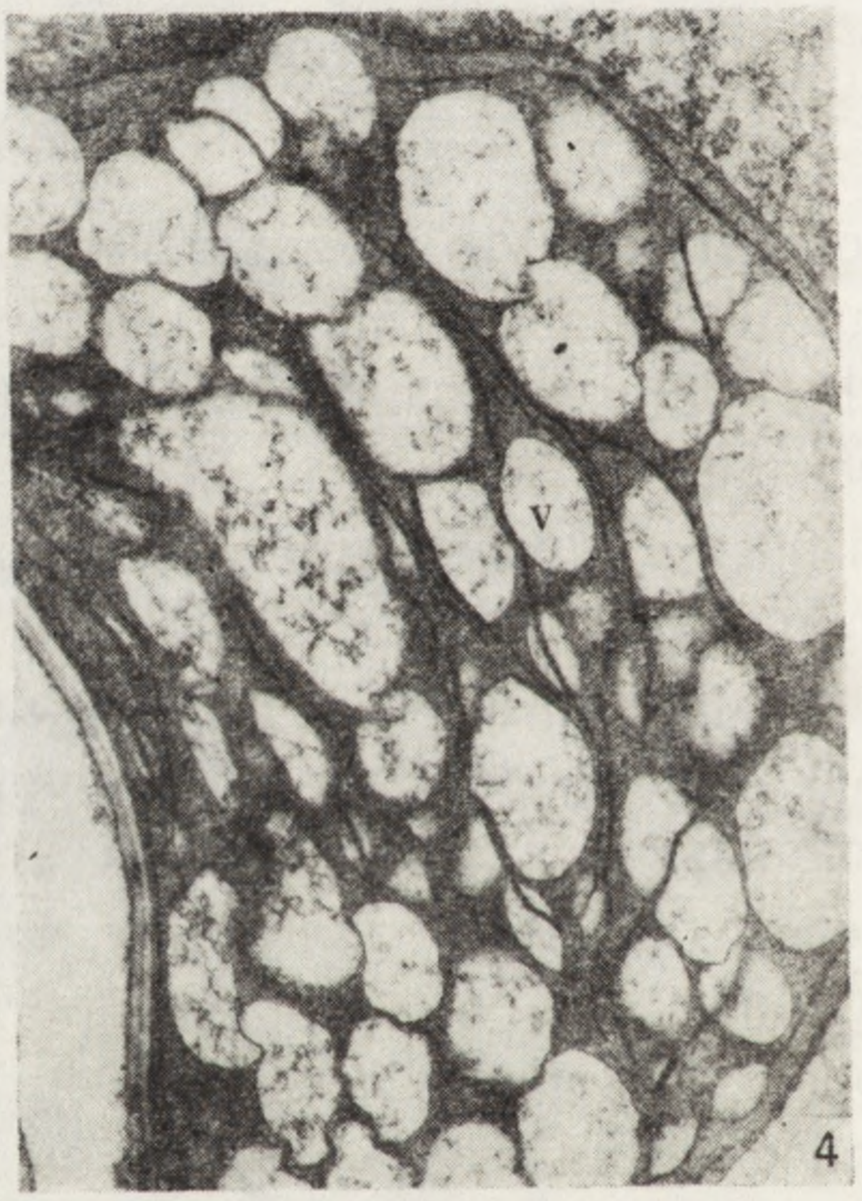


Fig. 1. Etioplast from 8-days-old etiolated maize seedlings (*Zea mays* L.)
Fig. 2. Chloroplast after 72 h of low intensity white light
Fig. 3. Chloroplast after 72 h of low intensity red light
Fig. 4. Chloroplast after 72 h of low intensity blue light
PB — prolamellar bodies, P — plastoglobuli, PT — perforated thylakoids, SG — starch grains, T — thylakoids, G — grana, ST — swollen thylakoids, V — vesicles

roplasts development. Transformation and recrystallization of prolamellar bodies occurs during the first few hours of blue light illumination. Stroma thylakoids differentiate. Thylakoids and especially their parts

are swollen. There are single vesicles. During further illumination numerous big vesicles are formed in addition to normally developed thylakoids. Prolamellar bodies are no longer on chloroplast sections after 24 h of light treatment. There are numerous and big vesicles in chloroplasts, paralell thylakoids are present on small areas of stroma after 72 hours. Prolamellar bodies and starch grains are not present (Fig. 4). These vesicles which are not present in normally developing chloroplasts of bundle sheath cells are most probably formed from numerous thylakoids swellings and their fragmentation.

III. Subsequent transformation and recrystallization of the prolamellar bodies take place at the first stage of chloroplast differentiation under continuous red low intensity light treatment. Already after 4 h and more clearly after 8 h light treatment, local thylakoid swellings and vesicles are visible on chloroplast sections. Vesicles are most numerous after 12 h of red light treatments. They are accompanied by very small and usually transformed prolamellar bodies. After 24 h of illumination the vesicles are usually no longer visible. There are few small paracrystalline prolamellar bodies and several 2–5 compartment grana (Fig. 3).

Finally, after 72 h of continuous low intensity light treatments chloroplasts without prolamellar bodies and vesicles differentiate. They have bundles of thylakoids with few swellings and some grana. Starch is not present. Continuous low intensity light treatment leads to formation of chloroplasts of a different structure, depending on the wavelength in bundle sheath cells.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (525—526)

THE EFFECT OF BIOLOGICAL ACTIVE SUBSTANCES IN THE RED BLOOD CELL MEMBRANES

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The effects of N,N,N-trimethylglycine chloride decyl ester (RW-315), N,N,N-trimethylglycine chloride dodecyl ester (RW-316), and N,N,N-trimethylglycine chloride 2-chlorethyl ester (RW-19) on the human erythrocyte membranes were studied. These compounds are known as a fungicidal and bacteriocidal active. Both compounds RW-315 and RW-316 cause hemolysis of the red cells at the milimolar range of concentrations studied (above 0.1 mM). Smaller than 0.1 mM concentrations of RW-315 and RW-316 were used in order to establish the influence of

TABELA 1

K values and hemolytic effect for RW-treated cells

Concentration (mM)	RW-315	RW-316	RW-19
0.0	0.0	0.0	0.0
0.001	7.8	2.1	2.2
0.01	11.8	1.2	5.2
0.05*	15.6	22.6	4.8
0.1	hemolysis	hemolysis	4.7
1.0			14.6
5.0			14.8

* Small hemolysis of the transport samples was observed.

these compounds on the sulfate exchange across the modified human red blood cell membranes. The determined rate constants for the exchange of sulfate anions across the control and modified cells were identical with control values within the range of experimental error (see Tab. 1). The third compound RW-19 does not cause hemolysis of the cells up to

5 mM concentration, and no change in the rates constant of sulfate transport were observed for cells treated with RW-19 (Tab. 1). The results of these studies indicate that these biological active substances influence the lipids of the membrane (observed hemolysis), but do not change band 3 protein which catalyses the anion exchange across the red blood cell membrane [1, 2].

The experiments were conducted with blood from healthy adult donors. Cells were incubated in the sulfate buffer solution pH 7.3 at 37°C, for 2 hrs. After that the red blood cells were loaded with 35_{SO4} tracer and treated with RW-compounds at concentrations (Tab. 1) at 37°C, for 2 hrs. After that the red blood cells were loaded with $^{35}SO_4$ determined according to the theory of tracer exchange in a closed two-compartment system [3].

By using the rate constants k_o (min^{-1}) for control cells, and rate constants k_m (min^{-1}) for modified cells, the constant $K = (1 - k_m/k_o) \cdot 100\%$ was formed. It is the percent change of k_m relative to k_o. The result of K calculations are summarized in Tab. 1.

The RW-315 and RW-316 compounds cause partial hemolysis of the cells at 0.05 mM concentration, but complete hemolysis at 0.1 mM. The hemolytic effect was not observed in the RW-19 case, even for 50-fold higher concentration. The results suggest that the presence of long 10 C or 12 C hydrocarbon chains in RW-315 and RW-316 respectively, determines their biological activity (RW-19 does not possess any chain). These compounds presumably have high affinity for membrane lipids, and can be easily incorporated into the membrane due to hydrocarbon chains. Similar effect was found in paper [4]. Possibly the incorporation of some critical numbers of RW-compounds molecules into the membrane creates big-enough channels for haemoglobin leakage. The studies of sulfate transport across the RW-treated membranes do not reveal any change in the rate constants for modified cells. These results show that the function of band 3 protein as an anion exchange catalyzer [1, 2] is not changed after the RW-compound treatment.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (527—528)

EFFECT OF SOME GLYCINE ESTERS ON SULFATE ION TRANSPORT ACROSS MODEL BIOLOGICAL MEMBRANES

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The present paper is a continuation of the studies on the molecular mechanism of action of some biologically active amphiphilic compounds on the liposome membrane that models the cell membrane. Both the parameters characterizing the hydrophilic parts of these compounds and the parameters which describe their hydrophobic parts have been studied. The following new group of compounds, i.e. glycine esters, was investigated:

— N,N,N-trimethylglycine chloride decyl ester
— N,N,N-trimethylglycine chloride dodecyl ester
— N,N,N-trimethylglycine chloride tetradecyl ester
— N,N,N-trimethylglycine chloride hexadecyl ester

These compounds show a strong fungicidal action.

The unilamellar vesicles were formed by using the cholate method with lecithin prepared in our laboratory from egg yolks. The transport kinetics of sulfate ions was studied by using the radioactive tracer method. The concentration of glycine ester in the liposome dispersion ranged from 0 to 6 mM, at pH = 7.5 and temperature 25°C. The rate constants for ion permeation across the liposome membrane were determined on the ground of kinetic curves by using a graphic method. When a dispersion of liposomes was treated with these glycine esters, the increase in the rate constant of sulfate ions transport was observed.

The rate constants increase with the increase of the glycine ester concentration in the dispersion. The increase depended on the chain length. The greatest changes were observed for trimethylglycine chloride tetradecyl ester, which has 14 carbon atoms in the alkyl chain, the smallest — for trimetylglycine chloride decyl ester which has 10 carbon atoms in the chain. The dependence of the rate constant upon the number of carbon atoms in the chain shows the maximum for 14 carbon atoms. The comparison of these results with analogous dependence obtained in previous experiments for homologous series of alkoxymethylene trimethylammonium chlorides shows that similar values of rate constant for sulfate ion permeation are obtained, in case of glycine esters, for a longer alkyl chain. It can be concluded, that alkyl chain of glycine ester in comparison with the alkyl chain of the same length of alkoxymethylene trimethylammonium chloride causes smaller looseness of the membrane hydrophobic layer. The strongly polar carbonyl group, which is placed near the ammonium group, at the beginning of the alkyl chain must be responsible for this situation. The carbonyl group, because of the interaction with the membrane polar layer, causes less deep incorporation of the chain into the hydrophobic layer. It seems that the insertion of additional polar groups at the beginning of alkyl chain could cause a weaker action of an amphiphilic compound on the lecithin membrane.

POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (529—532)

EFFECTS OF DIFFERENT SUSPENSIONS ON PHAGOCYTOSIS AND CYTOPLASMIC STREAMING IN *PARAMECIUM BURSARIA*

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Two motile activities of Paramecium cytoplasm, phagocytosis and cytoplasmic streaming, have not been studied in the same cells at the same time. Preliminary data have shown that particulate material (carmine particles) in the medium accelerate not only the rate of phagocytosis but velocity of cytoplasmic streaming too [7].

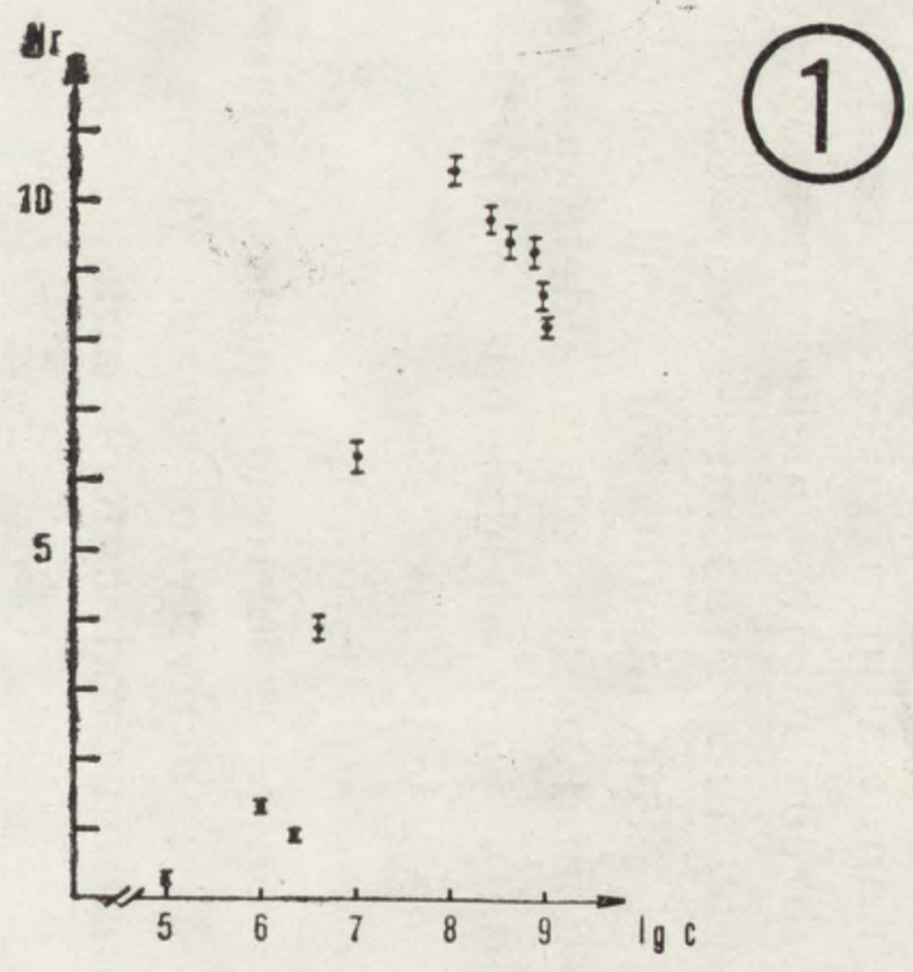
The question arises as to whether the nature of the particles or their concentrations are mainly responsible for the induction of phagocytosis and acceleration of cytoplasmic streaming velocity.

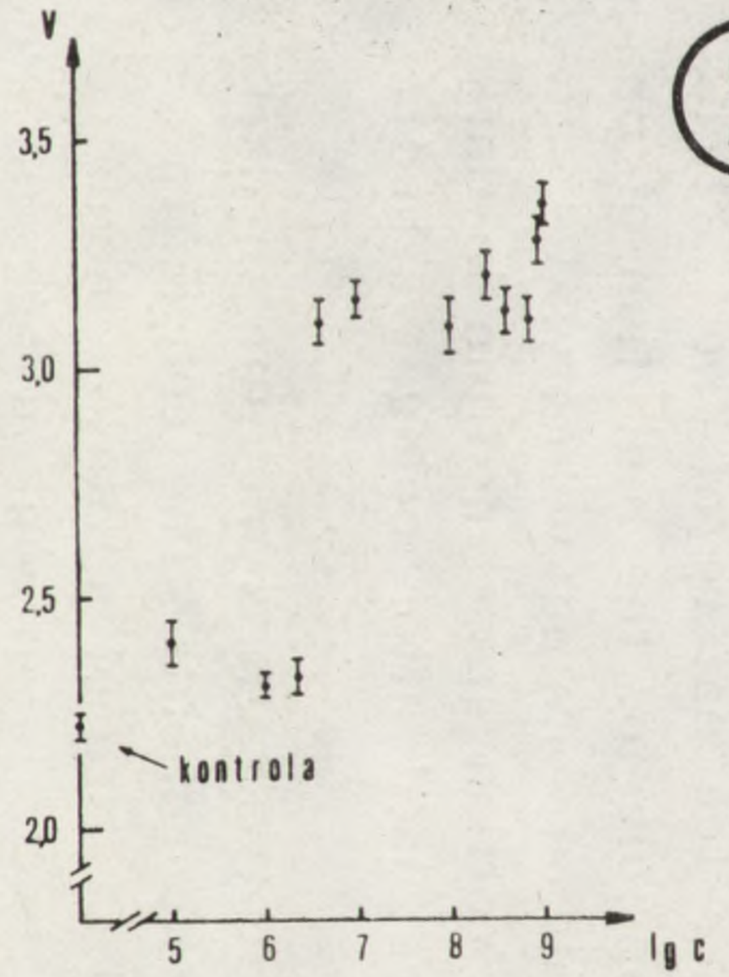
Paramecium bursaria grown on Scottish grass medium in darkness at room temperature (19 ± 2°C) were used after being washed overnight in maintenance solution [6].

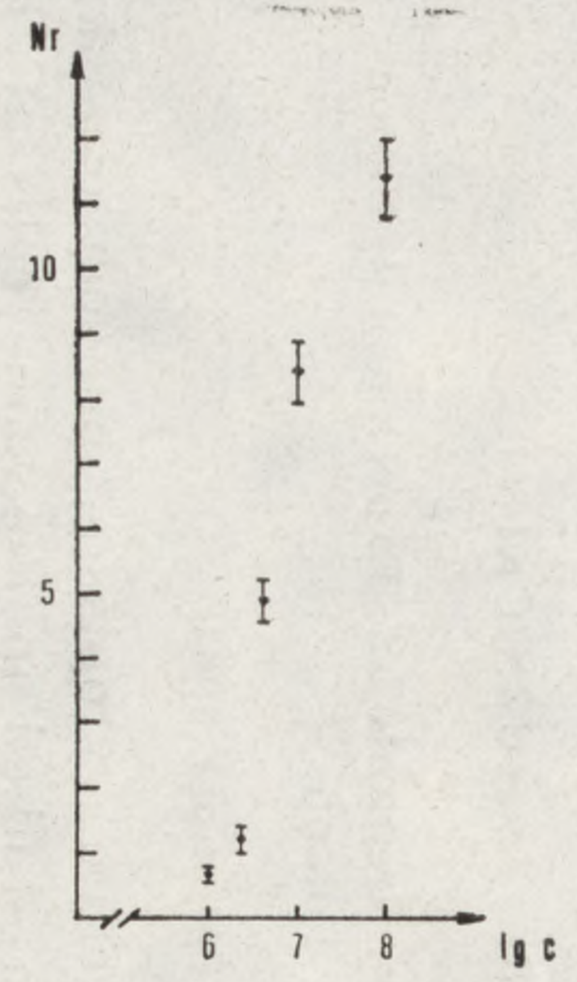
Paramecium bursaria grown on Scottish grass medium in darkness bacteria *Enterobacter aerogenes*, in experiments. The concentration of particles in suspension was estimated by use of a Bürker hematocytometer or by subsequent dilutions of bacteria grown on agar in Petri dishes.

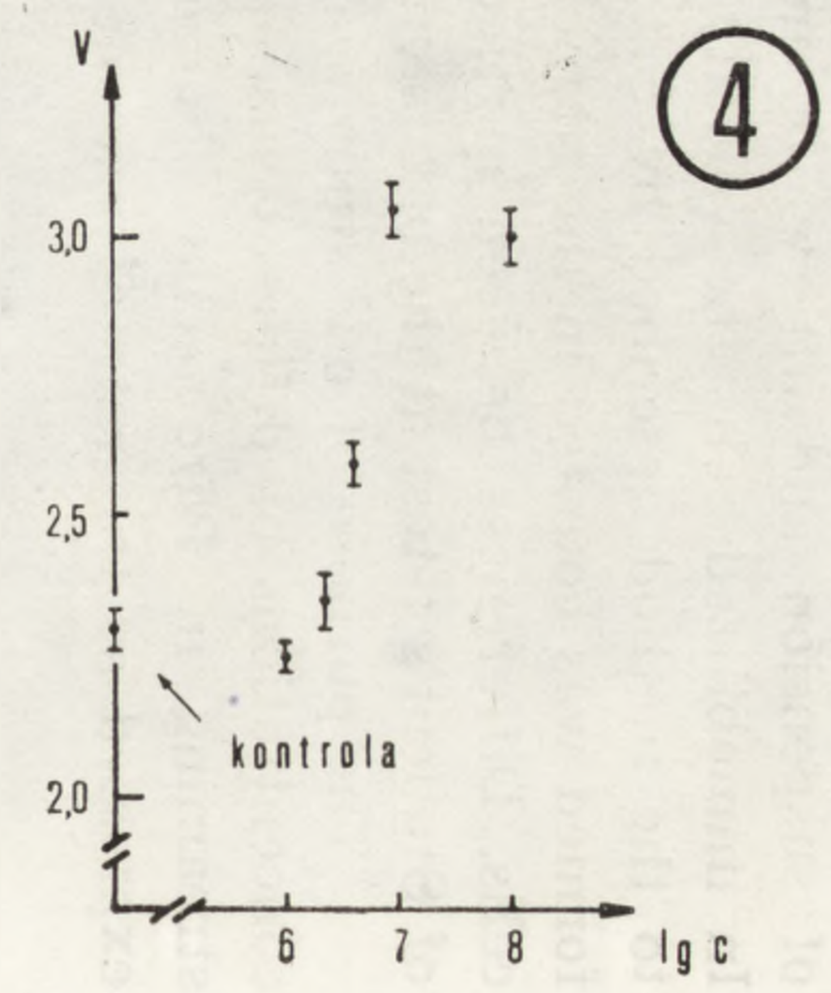
Paramecia were incubated for 3 minutes in a given concentration of suspension and afterwards immobilized by the nickel method [5]. In immobilized cells the velocity of streaming was measured according to the method described by Sikora [4]. The number of food vacuoles formed was counted in the immobilized and in the glutaraldehyde-fixed cells. Differences between particular data were evaluated on the basis of Student's t-test at the 0.02 significance level.

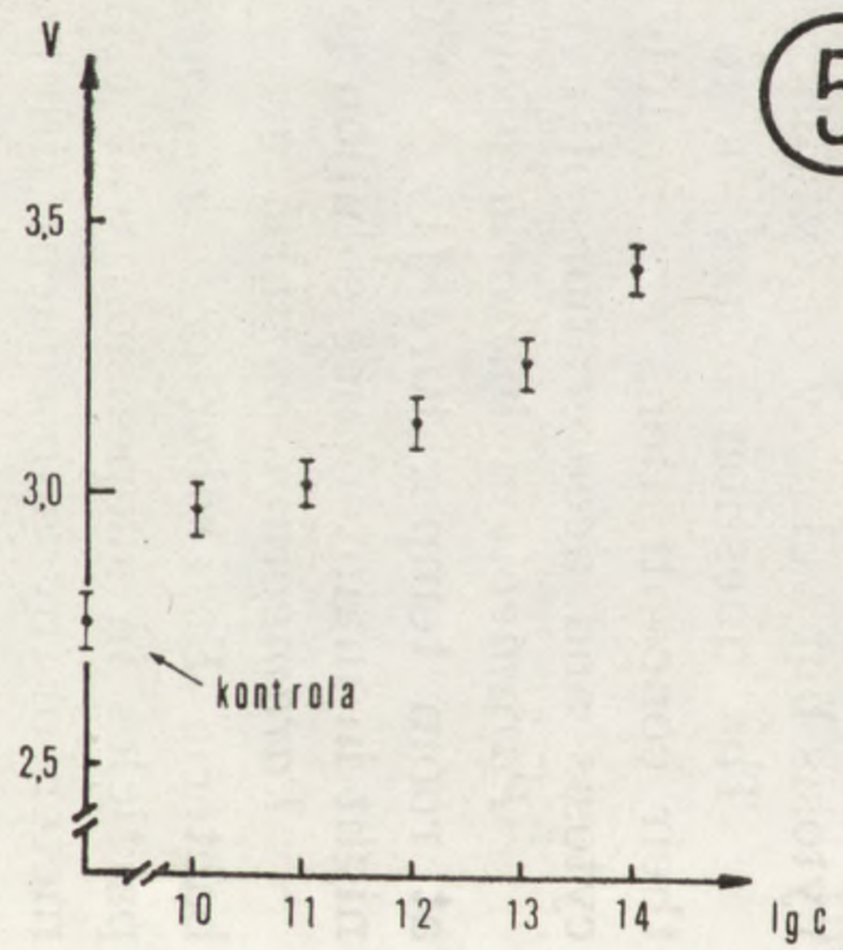
The purpose of our study was to estimate the influence of different concentrations of different suspensions on phagocytosis and cytoplasmic streaming in *Paramecium bursaria*. The activity of phagocytosis was expressed as the rate of vacuole formation, and cytoplasmic streaming











Influence of different concentrations *c* of carmine suspension (Fig. 1) and barium chromate (Fig. 3) on number of food vacuoles (Nr) formed in *Paramecium bursaria* cells. Influence of different concentrations *c* of carmine (Fig. 2) barium chromate (Fig. 4) suspensions, and bacteria *Enterobacter aerogenes* (Fig. 5) on cytoplasmic streaming velocity (v) in $\mu m\ s^{-1}$ in *Paramecium bursaria* cells. Mean value ± standard error

by its velocity. Paramecia exposed to low concentrations (1×10^5–$2.5 \times \times 10^6$ particles per ml (p.p.ml)) of carmine suspension almost do not form vacuoles (Fig. 1). The number of food vacuoles increased with a gradual increase of the concentration of carmine suspension. A distinct rise of the rate of food vacuole production occurred in concentrations over 2.5×10^6 p.p.ml. Phagocytosis reached its maximum at $1 \times \times 10^8$ p.p.ml (Fig. 1). No differences between number of food vacuoles formed in nickel-immobilized and glutaraldehyde-fixed cells were observed.

Cytoplasmic streaming velocity was measured in the same specimen after examination of food vacuole number. In low concentrations of carmine, *Paramecium* showed a slight increase in streaming velocity in comparison to the control specimens (Fig. 2). A considerable increase in cytoplasmic flow was observed in concentrations from 2.5×10^6 to $5 \times \times 10^6$ p.p.ml (Fig. 2).

The effect of barium chromate suspension on the rate of food vacuole formation and velocity of cytoplasmic streaming in *Paramecium bursaria* was similar to that of carmine (Figs. 3 and 4).

The influence of edible particles (bacteria) of about 1 μm in diameter on cytoplasmic streaming velocity is shown in Fig. 5. The range of concentrations which induced an acceleration of streaming was notably higher as compared to those at carmine and barium. These results might suggest that larger particles of 1.5–2.5 μm in diameter, as in case of carmine and barium chromate, are more effective in stimulation and increase the streaming velocity. Nevertheless, the general effect is much the same as that produced by nonedible particles (carmine and barium chromate).

The presented data confirm Rasmussen's [3] suggestion that particulate material is necessary to induce food vacuole formation and also confirm the influence of suspension on the rate of this process [1, 2].

Similar changes in the velocity of cytoplasmic streaming in *Paramecium bursaria* induced by increasing concentrations of different kinds of particles suggest that particulate material is substantially involved in the control of cytoplasmic motility.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (533—534)

CYTOSKELETON MOVEMENTS IN *AMOEBA PROTEUS* UNDER MODIFIED ATTACHMENT CONDITIONS

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Movements of different cell layers were examined by time lapse cinematography of amoebae normally attached to the glass surface, moving on the interface of water and fluorinert fluid, gliding on a layer of dense methylcellulose slime, and adhering to a solid but unstable substrate. Monotactic and polytactic forms of *A. proteus* were examined.

Monotactic amoebae laying on the fluorinert fluid or methylcellulose layer, what makes the attachment impossible, manifest a full fountain streaming. It means that the cell does not change its position, the endoplasm is flowing as usually forwards, but the ectoplasm moves backwards not only in the frontal zone but along the whole cell length. In other words, the whole ectoplasmic cylinder is retracted toward its closed rear end which represents its geometrical centre. Velocity of the ectoplasm withdrawal increases from the tail region to the frontal cap in linear way. It probably results from the general cortical contractility and summation of the contraction effects along the whole ectoplasmic tube with the growing distance from the centre of retraction. The ectoplasmic cylinder of monotactic amoebae attached to the glass surface is not retracted to its geometrical centre but is withdrawn from both sides toward the adhesion zone. Velocity of retraction also increases linearly from the attachment site toward both distal extremities. It produces as well the withdrawal of tail region behind the attachment area, as the fountain streaming in front of it. The fountain streaming is therefore based on the same mechanism — it is also a manifestation of the longitudinal component of the cortical contraction. It means that in the fountain not its axial endoplasmic arm is active, but the peripheral arm with the cortical layer.

In polytactic amoebae attached to the substrate the fountain may

appear only in the unattached frontal segments of leading pseudopodia. It was produced in pseudopodia guided upwards by shading their tips, in amoebae obliged to cross a ditch in the bottom of test-chamber, or migrating on a nylon tissue network. The backward movement of the frontal ectoplasm toward the adhesion sites ceases when the tip of free pseudopodium is allowed to establish a new attachment point. When the fluorinert fluid or methylcellulose layer prevent any attachment, the polytactic amoebae display as well the full fountain along their whole length, but in a different form. Together with the ectoplasm, the lateral pseudopodia and all other elements of the cell outline are also retracted toward the posterior body end, i.e. the whole cytoskeleton is "flowing" backwards. In the radial cross-section the movement of all elements is nearly uniform, and in the longitudinal the velocity changes are again linear. It should be concluded that also in normal polytactic cells the cytoskeleton is retracted toward its geometrical centre when suspended, or retracted from all sides toward the adhesion sites when attached and locomoting.

Therefore, probably a tension due to isometric contraction conditions should be developed between two or more different adhesion sites. It is confirmed indirectly by the behaviour of amoebae which attach to the unstable substrate: to short glass wool hairs loosely covering the bottom of test-chamber. When a new glass hair is "caught" by the front of amoeba, at the first stage it is drawn backwards toward the cell centre, it becomes thereafter stationary when adhering to the middle body part, and finally is pulled forwards together with the retraction of the tail region. Its movements strictly follow the cytoskeleton movements.

However, the use of other markers proves that simultaneously the whole surface material is normally flowing from the posterior cell regions toward the front of locomotion. It means that the movement upon the cell surface is bidirectional: in general the cell membrane of a migrating amoeba flows forward, whereas the material attached by adhesion-like mechanisms does not follow the membrane flow but is obeying the cytoskeleton movements. Probably it is a manifestation of the same phenomenon which in the tissue cells is described as "capping".

POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (535—538)

BEHAVIOUR OF *AMOEBA PROTEUS* IN THE GRAVITATION FIELD

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The influence of gravitation on the locomotion of *Amoeba proteus* has never been quantitatively studied. Certainly it is the way amoebae migrate, which makes difficult any quantitative experiments. The locomotion of amoebae, characterized by extension of new pseudopodia in different direction and retraction of the old ones, makes each individual hardly comparable to the others. A possibility of controlling the cell migration and the cell shape by reduction of the fronts number and determination of the locomotion direction has been provided by employment of the stripes of shade (Fig. 1). The method of steering amoeboid movement by the dark tracks seems to be appropriate to be used to study the geotactic response of amoebae in the quantitative way.

The specimens of *Amoeba proteus* which were taken for experiments came from the culture maintained in the *Pringsheim medium* and fed on *Tetrahymena pyriformis*. Only the well attached polytactic individuals were selected for the observations.

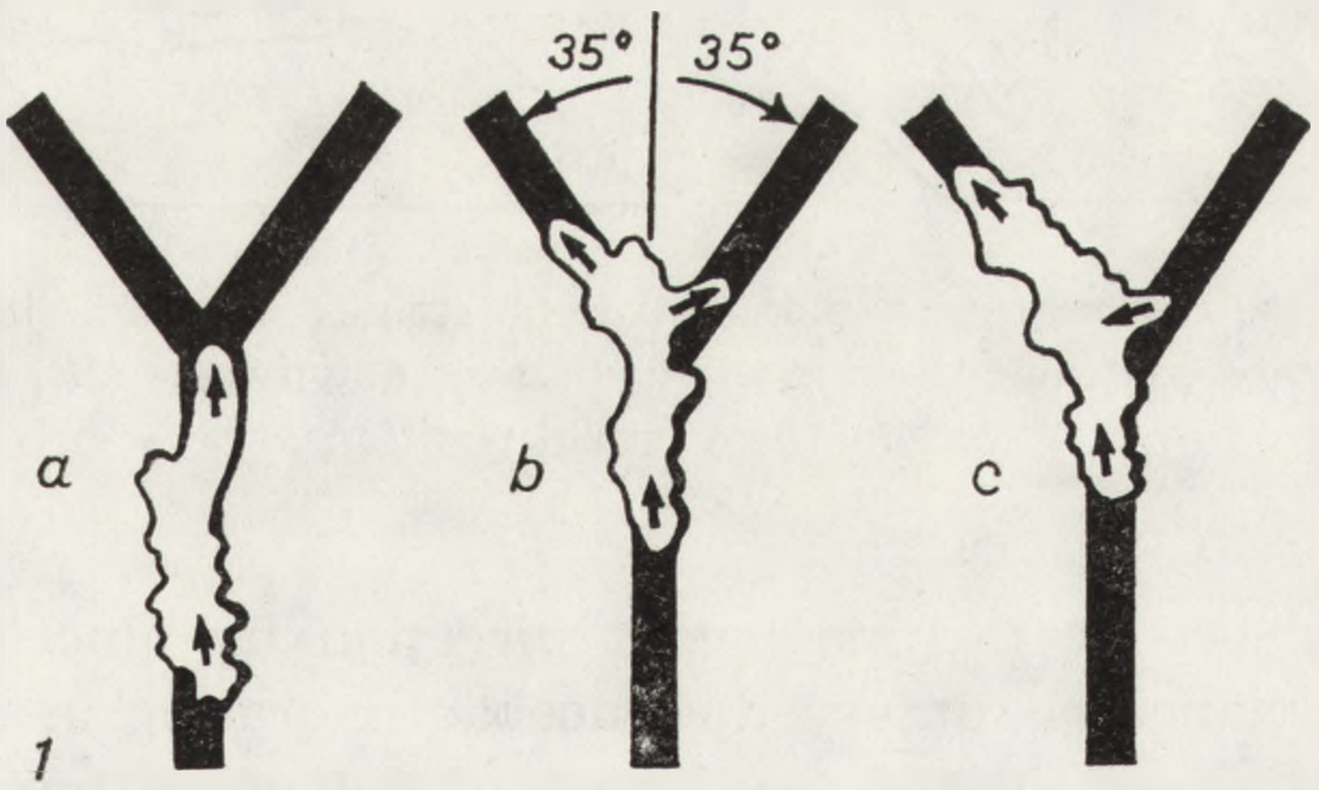
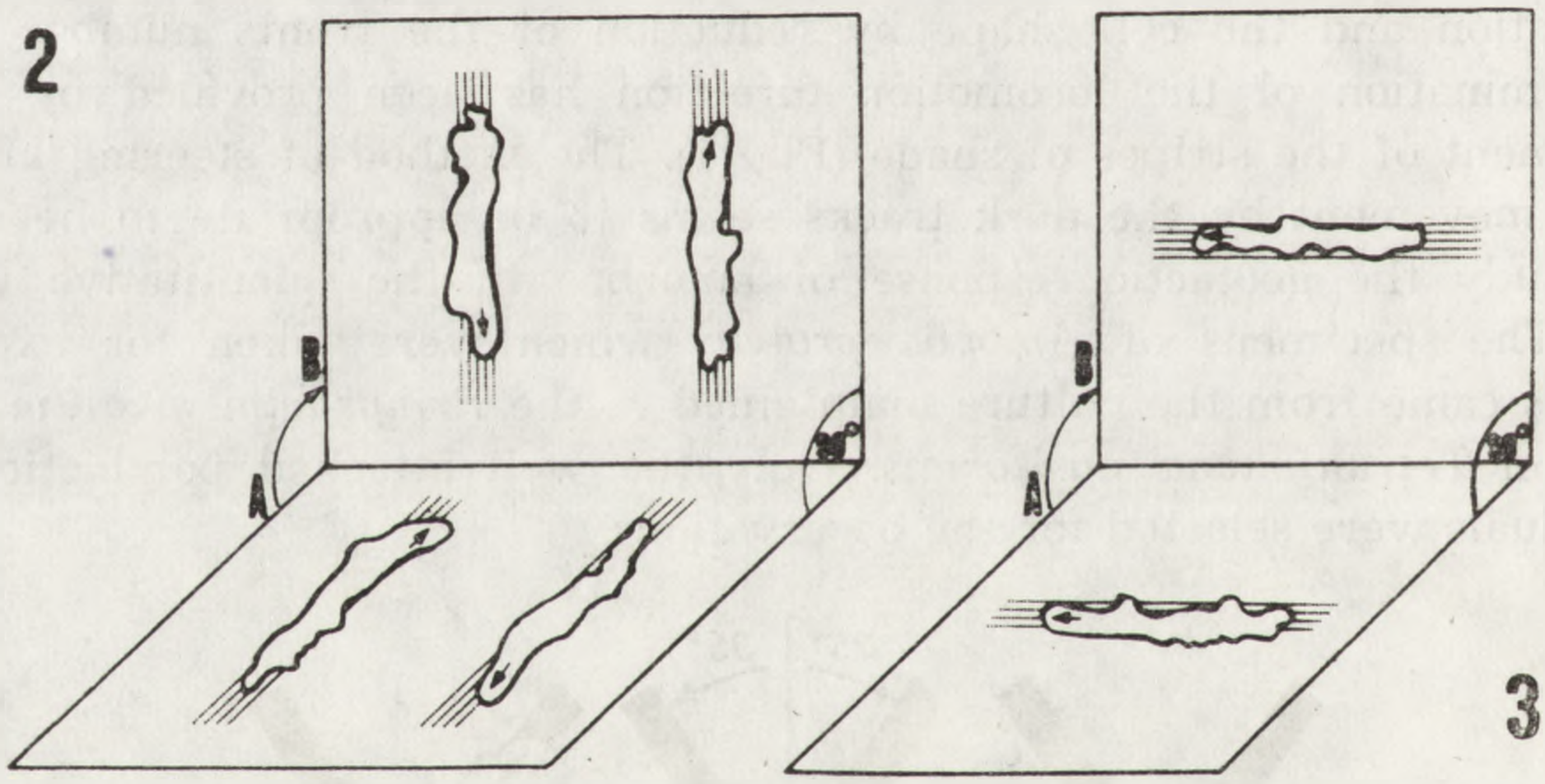


Fig. 1. Three stages of migration along the branching stripes of shade

All the experiments were run under the conditions in which the choice of direction in the horizontal plane was perfectly random. It means that there were no external stimuli which could influence the direction of movement. Because the observations of cell migration in the vertical plane were made under the identical conditions they guarantee therefore that only the force of gravitation were influencing the cell locomotion.

Velocities of locomotion along the straight dark stripes were measured in the horizontal and vertical plane. Amoebae were made to migrate along a dark stripe in the horizontal plane (Figs. 2*A* and 3*A*). When the position of the microscope was changed, together with the test-chamber, they continued to move along the same stripe but in vertical position (Figs. 2*B* and 3*B*). The cells migrate in the vertical plane, independently of the direction of their motion: upward, downward or transversally with approximately the same velocity as they move in the horizontal plane. The small velocity differences which were noted are not significant.



Figs 2 and 3. Amoebae migrating along straight stripes of shade in the horizontal plane (2*A*, 3*A*). They continue their motion upward, downward (2*B*) or transversally (3*B*) in the vertical position

Also the choice of the movement direction by amoebae migrating in the vertical plane was observed. Amoebae migrating in the horizontal plane were introduced in the basal part of a dark stripe in the shape of Y or T. When they became orthotactic (Figs. 4*A* and 5*A*) the microscope was turned by 90° (Figs. 4*B* and 5*B*). In each situation the final movement direction of 50 individuals was noted. In the studied popula-

thions the slight majority of amoebae always chose the upper branch of the shaded path.

It is concluded that *Amoeba proteus* manifests a statistically very weak tendency to the negative geotaxis. It should be, however stressed

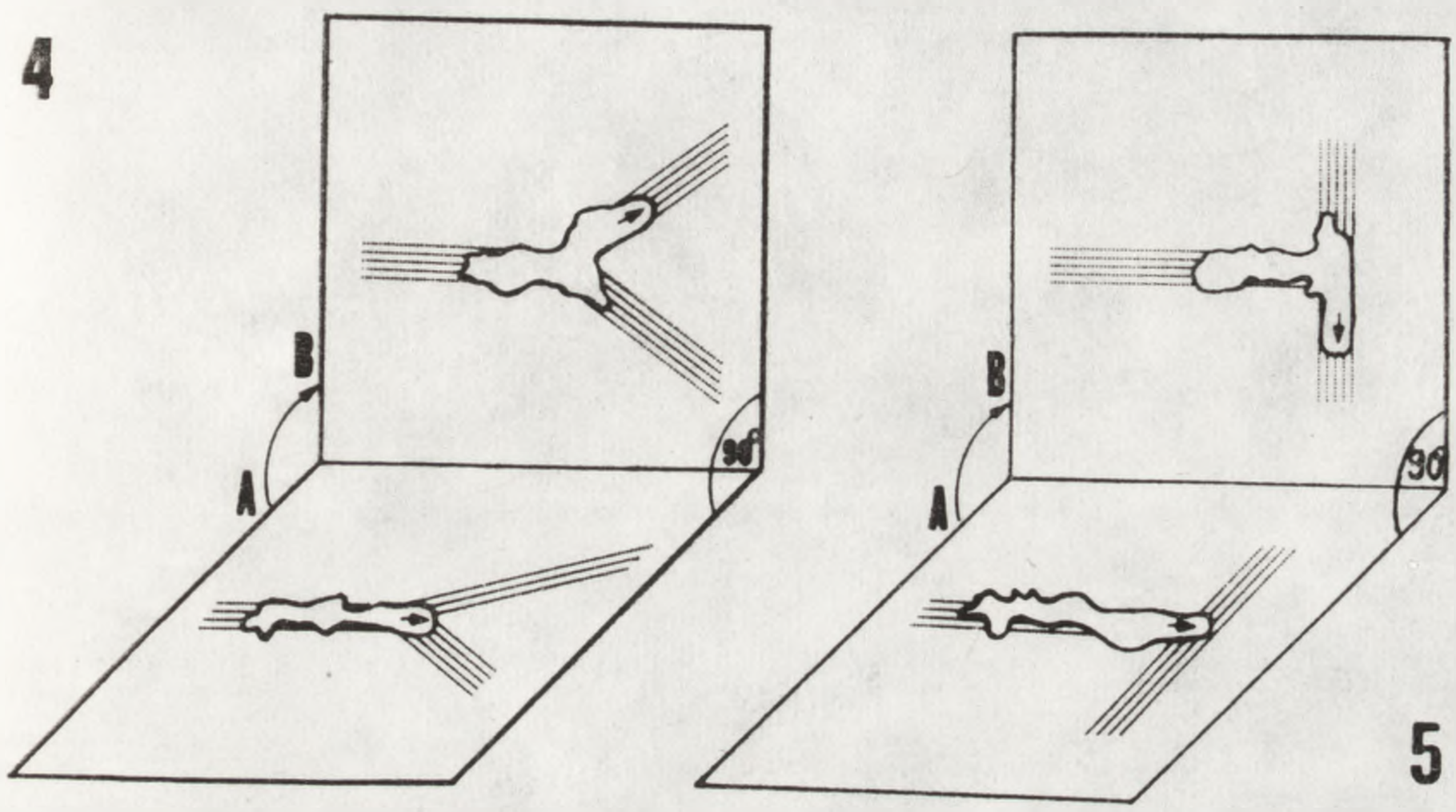


Fig. 4. Amoebae introduced into branching stripe of shade in the horizontal plane (*A*) and turned to vertical position (*B*)

Fig. 5. The same experimental situation as in Fig. 4, except the shape of the shaded path

that, although the vertical choice of direction by amoebae is not perfectly random, their response to the gravitation force is far from being as regular and uniform as in free-swimming *Ciliates* and *Flagellates*. It is also much weaker and probably different in nature from other taxes manifested by amoeba.

POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (539—542)

THE MICROBEAM SYSTEM AND ITS APPLICATION TO THE STUDY OF THE MOTILE BEHAVIOUR OF *AMOEBA PROTEUS*

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Several works on the effects of visible-light microirradiation of different parts of *Amoeba proteus* cells were previously published, but the authors applied a rather big (in relation to the cell size) light spot. Mast [4] used a rectangle 25 μm wide and 100 μm long; Saks [5] used a light spot 116–500 μm in diameter from a ruby laser.

We studied the motile behaviour of *A. proteus* induced by a small light spot (from 3 μm to 15 μm in diameter) obtained with the help of a homemade microbeam system. In our system the same microscope objective is used simultaneously for viewing the preparation and for the micro-irradiating (Fig. 1). For this purpose, an auxiliary lens system (3) focuses the light beam from the side halogen (100 W, 150 W) or HBO 200 lamps (*1a*) onto a small pinhole (*4*) (12.5 μm in diameter). The image of the pinhole is, in turn, reconstructed in the object plane of microscope with the help of the lens (*6*) (or pancratic objective) and microscope objective (*10*).

By suitably adjusting the position of the pinhole and the lens, it is possible to obtain a continuous change in dimension of the light spot.

In our experiments, we used well-attached and actively motile polytactic individuals of *A. proteus* on the 3-rd day after feeding. The amoebae were adapted for 15 minutes to very low viewing light intensity from an incandescent lamp, and then the active individuals were irradiated with a precisely localized microbeam of white or monochromatic light. All experimental data were compared with controls and tested by the χ^2 test.

For the white light spot we found that:

1. If only the hyaline cap was irradiated, no changes of streaming

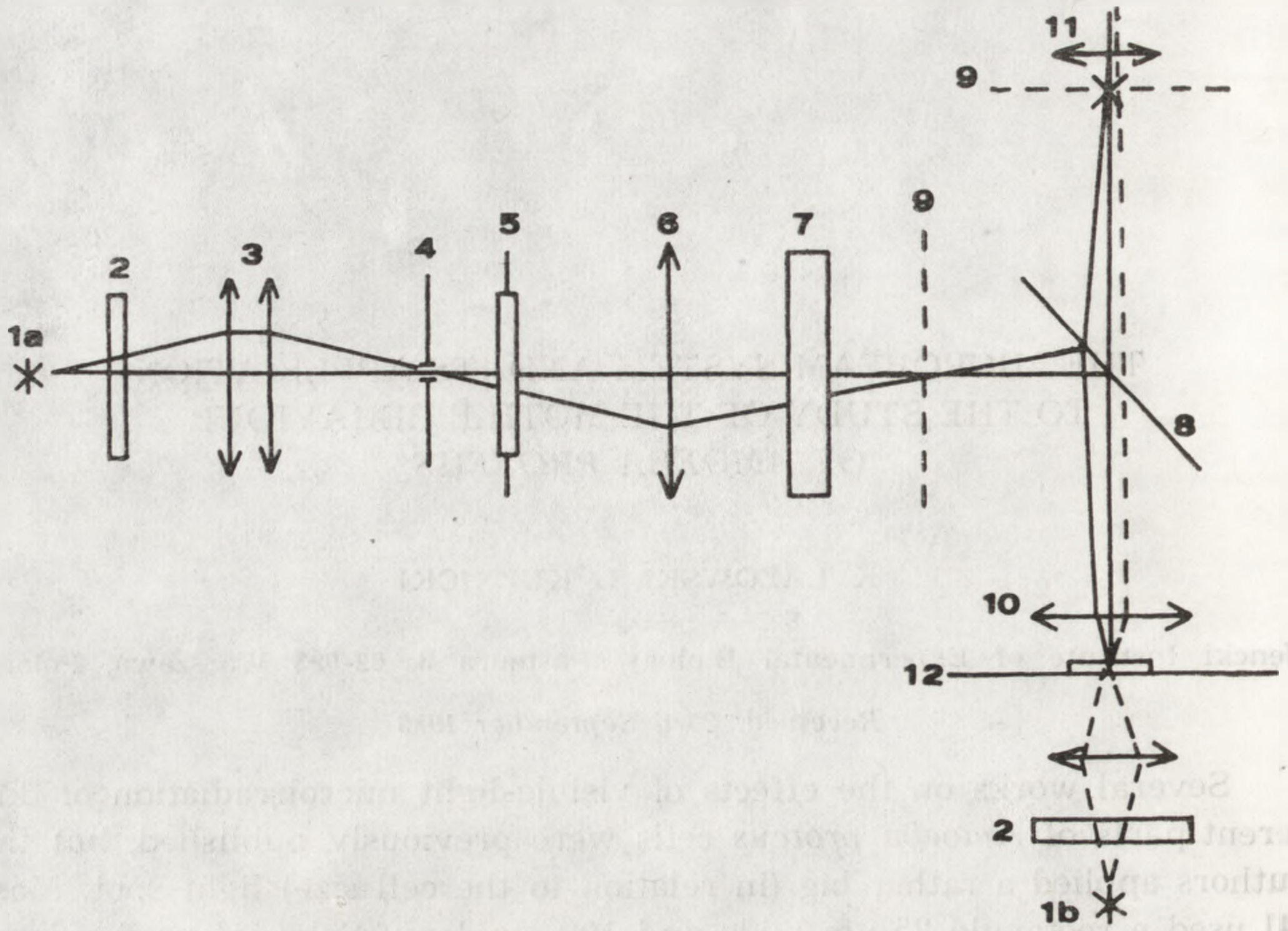

Fig. 1. The microbeam and viewing apparatus. Description in the text. solid line — denotes the irradiation system, dotted line — denotes the viewing (Biolar microscope) system; *1a, b* — light sources; *2* — warm filters W 302; *5* — shutter; *7* — interference filter holder; *8* — half-reflecting plate; *9* — intermediate image plane, and the pinhole image plane; *10* — microscope objective (10×, 20×, 40×, 160 mm); *11* — ocular with aiming cross hairs

Fig. 2. Modification of the streaming directions in monotactic *Amoeba proteus* after irradiation with white light; *a*) — before irradiation, *b*) — after irradiation; the black point denotes the place of irradiation and gelification

were observed until the tip of the plasmagel sheet was not illuminated with the spot. The same results were obtained by Mast [4]. To investigate this phenomenon, we also used the monotactic form of *A. proteus*, and we obtained the same data as above. When part of the plasmasol near the plasmagel sheet was illuminated, gelification at the place of irradiation and a modification of the streaming directions were seen Fig. 2.

2. If the tips of the plasmagel sheet of advancing pseudopods were irradiated, there was almost instantaneous cessation or reversal of streaming. In some cases, lateral pseudopods developed near the tip, or around the light spot; simultaneously, the streaming in other pseudopods continued.

3. If the base of advancing pseudopods or parts of the anterior were strongly irradiated, the streaming ceased and amoeba changed its body shape to a disc-like one; in some specimens the streaming reversed and the pseudopods were formed at the posterior end.

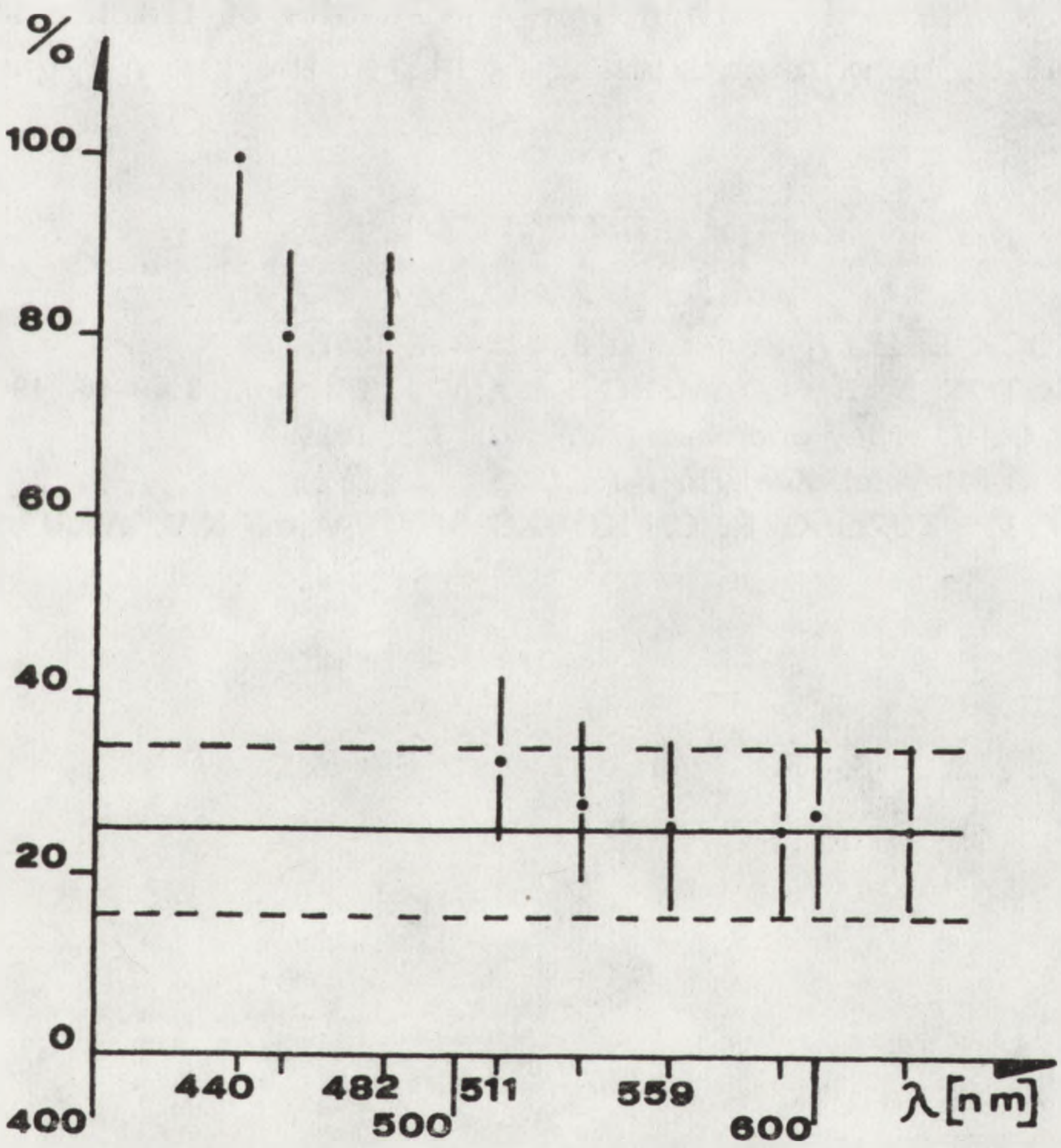

Fig. 3. The spectrum of streaming cessation in advancing pseudopods in politactic forms of *A. proteus*; ordinate — the percent of advancing pseudopods with light-induced streaming cessation, solid line — control, dotted line — standard deviation. Experimental points for 440, 453, 482, 511, 535, 559, 589, 600, 625 nm light

In the second experiment we reinvestigated the sensitivity of *A. proteus* to light of different wavelengths with the use of our microbeam system and Zeiss Metal-Interference filters, it was possible to illuminate monochromatically only parts of the plasmagel sheet which, as was found, is the part of the *A. proteus* cell most easily influenced by light. These results could be connected with the dynamic equilibrium state between plasmagel and plasmasol, at the hyaline layer interface in advancing pseudopods.

We found (Fig. 3) that blue light in the range of 440–482 μm has a very distinct effect on the streaming in pseudopods in comparison to control (χ^2 test). It produced a cessation of streaming in advancing pseudopods and gelification of the plasmasol near the plasmagel sheet. Light in the 511–625 μm range has only slight, if any, effect on streaming and gelification.

The results obtained in this experiment support the general conclusions of Harrington and Loaming [2] and Mast [3] which indicate a very marked effect of blue (430–490 μm) light on the rate of streaming in *Amoeba proteus,* rather than the results of Hitchcock [1], which indicate that the maximum sensitivity lies in the green region (515 μm).

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (543—546)

THE ROLE OF MECHANICAL STIMULI IN THE CONTROL OF CYTOPLASMIC STREAMING IN *PARAMECIUM BURSARIA*

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Considerable information is now available on cytoplasmic streaming in *Paramecium* (for review see [1]). Studies on this intracellular activity require the arrest of cellular locomotion but invariably have been hindered by the immobilization procedure. Recently, a most promising method has involved nickel-chloride induced ciliary paralysis [3]. Previous observations have suggested that *Paramecium* immobilized by use of this method exhibit cytoplasmic streaming is almost undisturbed in comparison with intact cells; however, there was lack of direct evidence supporting this supposition.

In view of recent findings [2, 4] on the possible role of particulate material in the regulation of cytoplasmic streaming velocity and the rate of phagocytosis, it was of interest to investigate these processes in *Paramecium bursaria* immobilized mechanically by means of microneedles.

Specimens of *Paramecium bursaria* cultured and handled according to Wasik [4] were anchored by means of two glass micromanipulator microneedles. Cells with needles inserted and demonstrating undisturbed cytoplasmic streaming were selected for the study. Cytoplasmic streaming velocity was estimated as previously described [1]. A suspension of carmine particles of 1.5–2.5 μm in diameter and in concentration (2.5–5 × 10^6 particles per ml) sufficient to induce distinct acceleration of cytoplasmic streaming velocity [4] was used to stimulate anchored cells.

The means of 5–7 sequential measurements of cytoplasmic streaming velocity taken at 2-minute intervals are shown in Fig. 1. Control paramecia in maintenance solution demonstrated no change of streaming velocity. The obtained values for control paramecia were similar to the

velocity of streaming described in nickel-inmmobilized *Paramecium bursaria* [5].

In our experiments (Fig. 1) a suspension of carmine particles was injected into the vicinity the cell held by means of microneedles. The suspension of carmine particles induced an increase of cytoplasmic streaming velocity in *Paramecium bursaria*, with the most distinct increase detected in the first minutes after exposure to carmine. Cytoplasmic acceleration lasted for 10–12 minutes; after that some fluctuation of streaming velocity was observed. However the range of velocities corresponds well with effects induced by a carmine suspension (in concentration 2.5–5 $\times$ 10^6 particles per ml) in nickel-immobilized *Paramecium bursaria* [5].

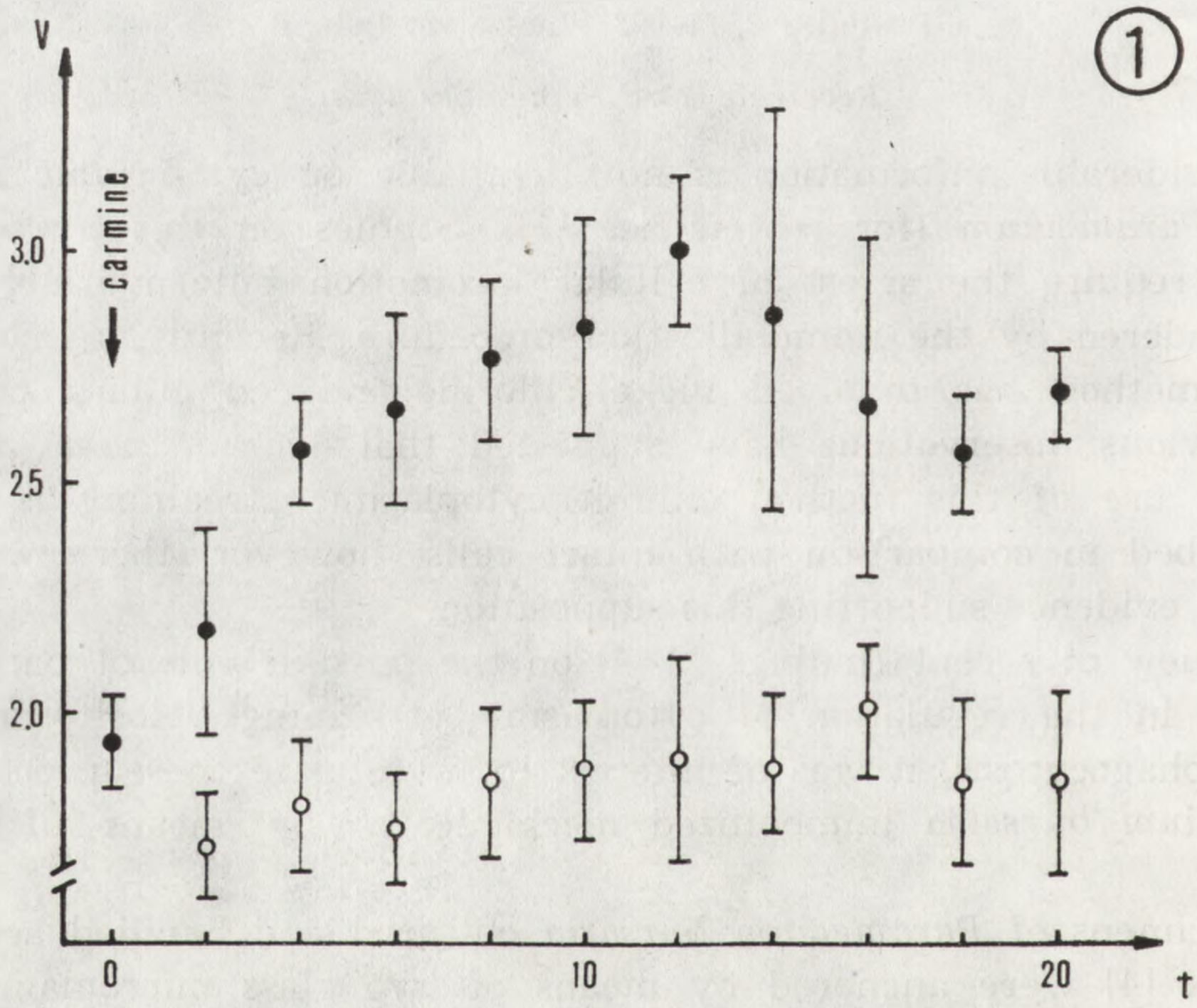

Fig. 1. Mean values ± S.E. of cytoplasmic streaming velocity (ordinate) in μm s^{-1} of *Paramecium bursaria* held by use of microneedles. Abcissa — time in minutes. Open circles — unstimulated specimens in maintenance solution. Closed circles — stimulated with carmine suspension in concentrations of 2.5–5$\times$$10^6$ particles per ml

One of the astonishing incidental observations was that puncture of *Paramecium bursaria* cell with microneedles (far from the cytostome) caused wounding of the cortex, which persisted at least 20–30 minutes and resulted in subsequent inhibition of food vacuole formation.

The time for observation and measurement of cytoplasmic streaming in nickel-immobilized *Paramecium* is limited. Routine procedures for immobilization of *Paramecium bursaria* have not permitted us to observe the intracellular events in nickel chloride treated cells until at least 13 minutes (extending to about 45 minutes) after beginning the treatment. Thus, there was no information on the dynamics of cytoplasmic streaming during the very first minutes after exposure to particulate material. Mechanical arrest has revealed that cytoplasmic streaming reaches its highest velocity (about 2.7 $\mu m\ s^{-1}$) within 10–12 minutes if stimulated with carmine suspension in concentrations of 2.5–5×10^6 particles per ml. The range of velocities recorded corresponds well with those exhibited by the nickel-immobilized paramecia after stimulation with carmine particles in the concentrations indicated above [5].

Unanswered questions concern the extent to which the velocity of cytoplasm is affected by microneedle puncture. A comparison of cytoplasmic streaming velocity induced by particulate material in the medium mean values for nickel-immobilized cells ($2.22 \pm 0.49\ \mu m\ s^{-1}$ S.D.) suggests that mechanical wounding has little effect on cytoplasmic streaming velocity. However, the possibility that both kinds of immobilization procedures may influence the streaming velocity cannot be excluded.

If food vacuole formation is inhibited, the acceleration of cytoplasmic streaming velocity induced by particulate material in the medium is of far reaching importance. It supports the idea that food particles directly stimulate the cell to provide membranous material for food vacuole formation and for transportation of newly formed ones. In view of the results presented, it is likely that mechanical stimulation, provided by solid particles in the medium, is a distinct factor in the control of cytoplasmic streaming dynamics in *Paramecium* cells.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (547—550)

TEMPERATURE DEPENDENCE OF OSCILLATORY CONTRACTION ACTIVITY IN *PHYSARIUM POLYCEPHALUM*

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The temperature of the environment strongly influences the behaviour of *Physarum polycephalum* plasmodia [7]. One of the factors, which determine motility of the plasmodia is their contractile activity. It was found that the frequency of contraction-relaxation cycle of plasmodial actomyosin increases with an increase in temperature [8, 9]. However, in order to determine the relationship between temperature and energy of contractile activity the frequencies, amplitudes and levels of the oscillations have to be analysed simultaneously. The aim of the present work is to determine a qualitative characteristic of the changes of energy of contractile activity in relation to variations of temperature.

The contractile activity of isolated plasmodial strands and endoplasmic veins [1] has been studied with the aid of a tension transducer [8]. The samples were submerged in salt solution [4]. The temperature of solution was controlled by means of a Peltier element and changed gradually (about 4°C per 10 min) within the range of 12–28°C. According to the signal theory [3] energy of a signal is defined as follows: $\int_{-\infty}^{+\infty} X^2(t)dt$. Tensiograms $F(t)$ were taken as a signal $X(t)$. The evaluated tensiograms were integrated within 20 min range.

It was found that the temperature dependence of the oscillation period (T) in the physiological range of 16–28°C [9] is greatly approximated by the function $T = \frac{a}{^{o}C} + b$ (Fig. 1 and 2). The slope a might change depending on the sample in the range of 40–60 (in coordinates shown in Fig. 2). At temperatures below 16°C, a decrease of the slope is occasionally observed (Fig. 2). In contrast to frequencies, a simple temperature dependence of amplitudes or levels of oscillations was not found. However, a tendency to an increase of both parameters with a decrease in temperature was observed (Fig. 1). This observation suggests that the

energy of contractile activity of the samples might increase in lower temperatures. Table I shows an example of an almost threefold increase of the entire energy E and the energy of the contraction-relaxation cycle E_{osc} with a gradual decrease in the temperature from 28.8°C to 11.5°C.

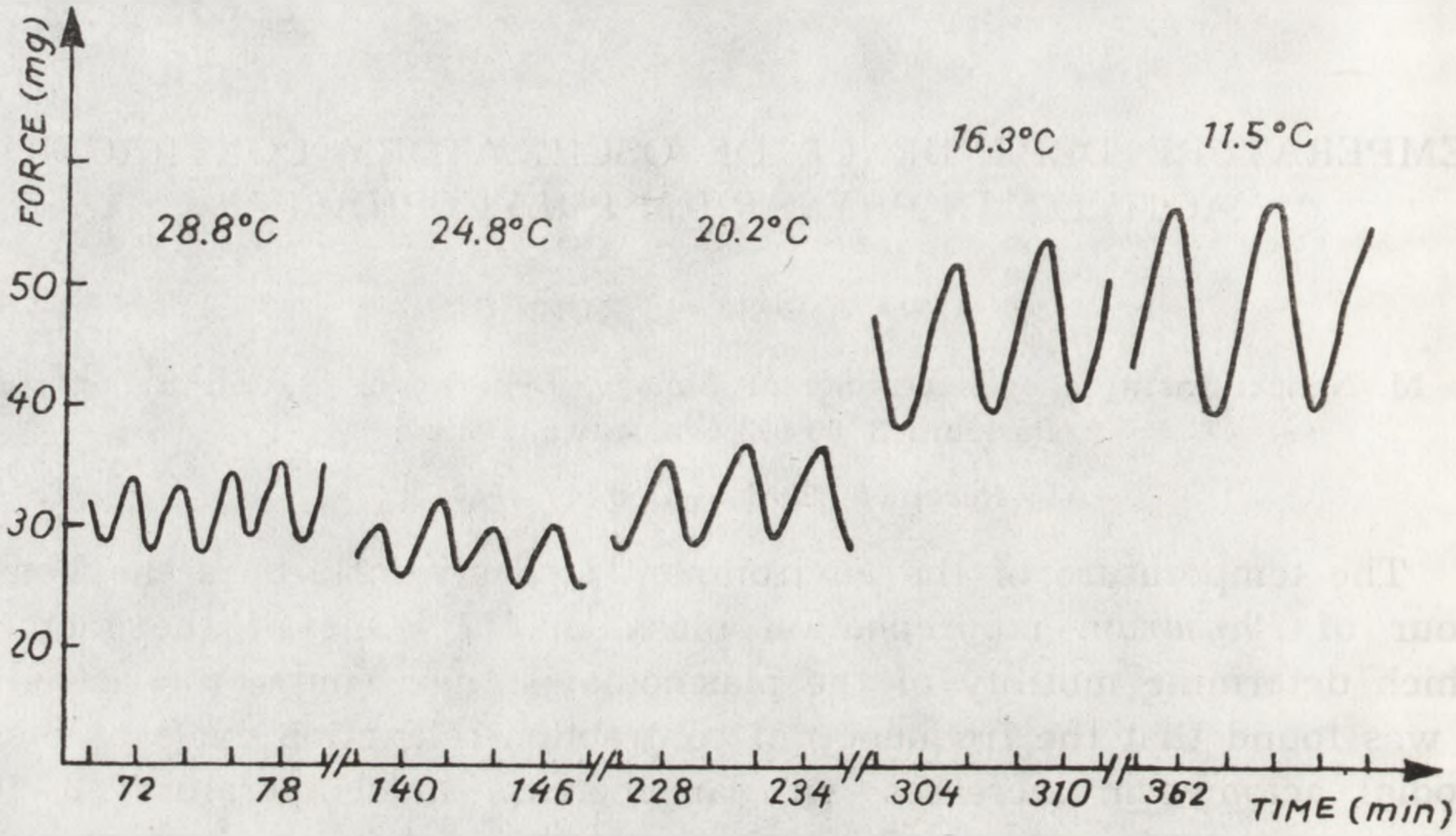

Fig. 1. Changes of contractile activity of a plasmodial strand in response to a gradual decrease in temperature. Abscissa: time course of experiment, ordinate — force in milligrams

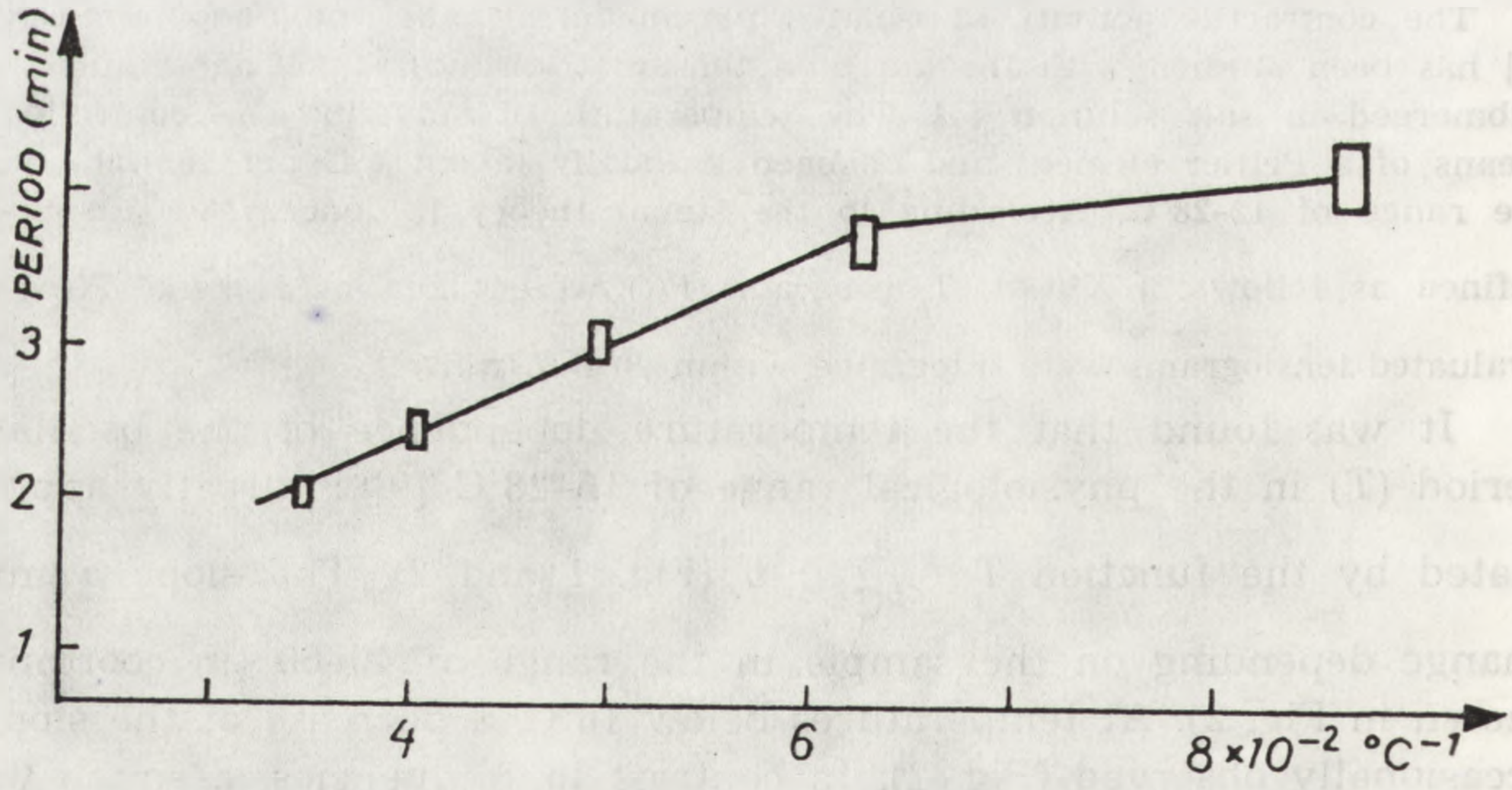

Fig. 2. Temperature dependence of the oscillations period (T) obtained on the basis of the tensiogram, parts of which are shown in Fig. 1. Abscissa: inverse temperature, ordinate: period of oscillations (T)

TABLE 1

Changes of energy of contractile activity of a plasmodial strand with variation of temperature. The values are calculated on the basis of the tensiogram parts of which are shown in Fig. 1. E – entire energy, E_{osc} – energy of contraction-relaxation cycle (integration limited to minima of oscillations), where: $E = E_{osc} + \text{const}$

Temperature °C	$E \pm 0.5\%$ (in relative units)	$E_{osc} \pm 0.5\%$ (in relative units)
28.8	0.47	0.10
24.8	0.40	0.08
20.2	0.49	0.12
16.3	1.06	0.30
11.5	1.04	0.35

Recent data strongly suggest that the contraction-relaxation cycle of plasmodial actomyosin is controlled by energy metabolism [4] and that mitochondria play a substantial role in this control [2, 5, 6]. The obtained results on temperature dependence of oscillation frequency show that the energy metabolism precisely regulates the frequency of oscillations, while the control of the amplitude and the level of oscillations is less pronounced.

The relation between temperature and energy of oscillations support the importance of the contractile apparatus in the modulation of the level and amplitude of oscillations. An increase of the energy of contractile activity with a decrease in temperature implies an increase in the efficiency of the contractile system in lower temperatures. We suggest that this phenomenon is related not only to the shift of the G-F actin equilibrium in the direction of the filamentous state [10], but also an increase of affinity of myosin to actin. The results presented suggest that in plasmodia of *Physarum polycephalum*, the filamentous state of actin and the interaction of myosin with actin are controlled by the active inhibition of both processes.

Acknowledgement. The authors wish to thank Prof. K. E. Wohlfarth-Bottermann and Alexander von Humboldt Stiftung for their support giving tensiometer and temperature control devices.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (551—552)

ANALYSIS OF MOVEMENT OF THE FRAGMENTS SEPARATED FROM PLASMODIUM OF *PHYSARUM POLYCEPHALUM*

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Plasmodium of *Physarum polycephalum* serves for nearly 50 years as a suitable model for studying motility phenomena. Although the molecular basis of the protoplasm shuttle streaming is known in details, the mechanism of the eye-visible movement of the whole is still controversial. The main points of discussion touch the problem of the motive force localization. Whether it is situated in the network of channels or in the frontal zone, or everywhere — the question is still unsolved. The present experiments tend to add several facts which will help to elucidate the problem.

The small pieces of plasmodial mass were put in Petri dishes on non-nutrient agar. The examination started 48 hrs later. There were distinguished two main regions in the topography of plasmodium: the frontal part (frontal margin + continuous protoplasmic layer) and the rear part = network of veins. These regions were separated from each other by cutting off in different configurations (Fig. 1). Time-lapse filming documented macroscopically the situation before and up to 15 min. after the operation. The speed of the frontal margin motion and the pulsation rhythmicity of the veins, respectively, were compared in the untouched plasmodia and after setting certain regions aside. In the second group of experiments the role of the frontal protoplasmic layer in the progressive movement was examined. The series of diverse cuttings were photographed every 20 min. and the observations lasted up to 3 hrs.

It can be concluded that the synchronal pulsation of the veins is widely spread in plasmodia of different shapes. The cuttings usually did not have any influence on the synchrony but if it happened they had, it was only the improving effect. The experiments under time-lapse filming revealed that the simple relationship and the synchronism between the veins and the frontal margin pulsation is not quite a com-

mon event. Long lasted observations seem to explain this fact. They point that the role of the continuous protoplasmic layer may be deciding in controlling the endoplasm influx or efflux from the adjoining segments of the frontal margin. It is supposed that the most important pushing role of the main veins of the network is combined with the modifying role of the continuous protoplasmic layer and the streamlets newly arising in it.

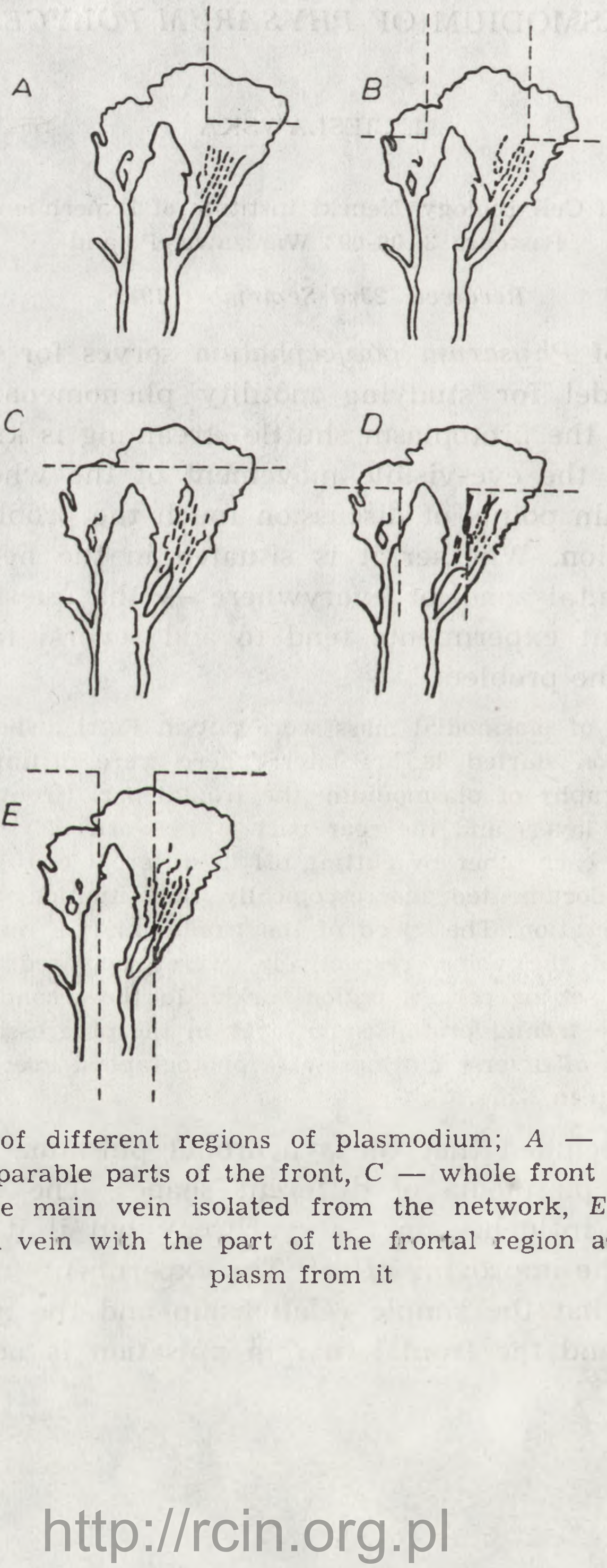

Fig. 1. Separation of different regions of plasmodium; *A* — 1/2 of the frontal part, *B* — two comparable parts of the front, *C* — whole front separated from the rear part, *D* — the main vein isolated from the network, *E* — prevented the contact of the main vein with the part of the frontal region accepting the protoplasm from it

POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (553—556)

SPECULATION ON THE ORIGIN OF TWO PHOTORECEPTION SYSTEMS IN *EUGLENA*

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It is known that sudden changes of light intensity evoke photophobic responses in *Euglena gracilis* (Fig. 1). These responses (step-up upon an increase and step-down upon a decrease of light intensity) are in close relationship, however there is an open question how many photoreceptor systems are involved. Mast and Hawk [2] and Shettles [5] studying the behavious of *Peranema trichophorum*, found that this colorless flagellate is sensitive to the light. Recently, Mikołajczyk [4] reported that at certain conditions, *Astasia longa* (Fig. 2), a mutant of *Euglena gracilis* lacking chloroplasts, stigma and blue light photoreceptor — paraflagellar swelling (PEB), responds to an increase of light intensity. In *Astasia cells* a photosensitive pigment seems to be the only pigment responsible for the step-up response, while in green flagellates possessing a highly specialized photoreceptor — PFB, it acts as an "accessory" one [3, 4]. In another words, sensitivity to increase in light intensity is not only limited to organisms possessing a highly specialized photoreceptors, like PFB [4]. On the basis of recently obtained results [3, 4] we postulate, that the step-up photophobic response in colorless flagellates is the most primitive photoreaction to a sudden increase of light intensity. The only biological meaning of this response is to avoid possibly harmful light effects. This light-orienting mechanism is probably originated from flagellates ancestors. The step-up and step-down photophobic responses associated with the paraflagellar swelling and stigma had developed as a secondary photoreception system. For *Euglena gracilis*, a photosynthetic organism this light sensory system is necessary for light-seeking purposes. Our hypothesis is in full agreement with the ideas of Kivic and Walne [1] who postulate that in autho-

trophic flagellates photoresponse mechanism is of relatively recent origin and represents numerous parallel evolutions. According to the authors, in the *Protista* kingdom, the light-antennae show the greatest phylogenetic variation, whereas the signal-processors and motile elements resemble those of other sensory system.

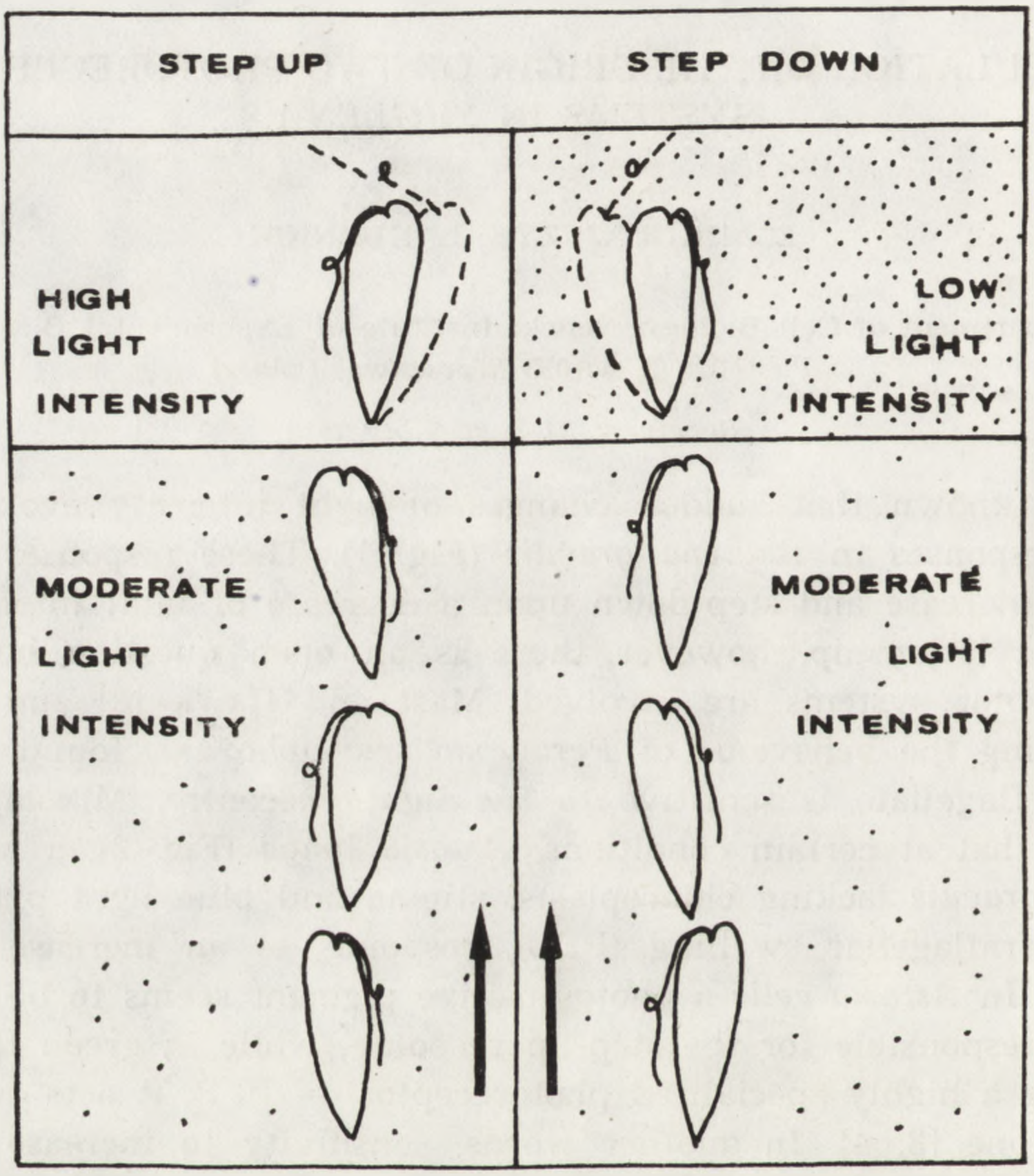

Fig. 1. Scheme of photophobic responses of *Euglena gracilis*. Arrows—direction of swimming

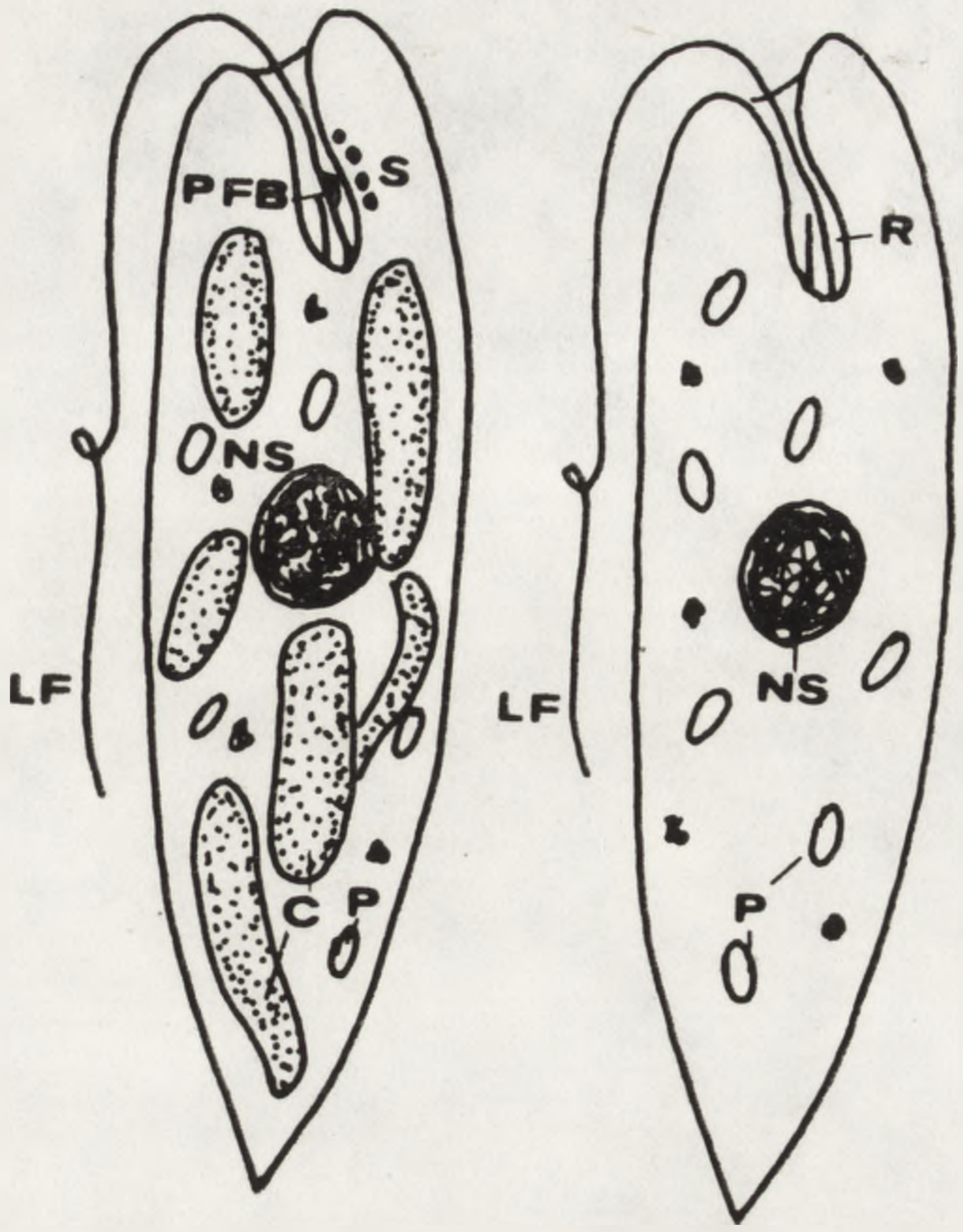

Fig. 2. Internal view of *Euglena gracilis* and *Astasia longa*. R — reservoir, PFB — paraflagellar swelling, NS — nucleus, LF — locomotor flagellum, P — paramylon

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (557—558)

THE INFLUENCE OF SOME ENVIRONMENTAL FACTORS ON THE MOTILITY OF *PSEUDOMONAS FLUORESCENS*

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The environmental parameters such as temperature and humidity are the main factors influencing the growth and the metabolism of the cells. They were taken into consideration in the studies on the influence of some halides on the motility of *Pseudomonas fluorescens*.

The observations were carried out in the dishes incubated at 37, 20 and 4°C. The motility at 37 and 20°C was visible after half an hour. After 3 hours it was possible to observe the effect of 1% NaF as petite zones of motility in comparison to control. Inhibition of motility of *Pseudomonas fluorescens* was evident when the dishes covered by semisolid agar with 0.1 M or 1% NaF and incubated at 4°C. The motility appeared after 72 hours in semisolid agar containing 0.1 M NaCl.

TABLE 1

Investigations on the motility of *Pseudomonas fluorescens*

	Medium	Size of colonies with the zone of motility			
		after 5 hours		after 22 hours	
		A	B	A	B
	Control	3—4—5	2—2.5—3	5—6—9	3.5—4—5
I	+1 mM $MgCl_2$	3—3.5	2—3—4	5—6.5—7	4—5<
	+10 mM $MgCl_2$	4—5<	3.5—4—4.5	6—9<	5—6<
	Control	1.5—2—3	1.2—1.5—2	3—4—6	2—2.5—3
II	+10 mM NaF	0.8—1—1.5	1—1.5—2	2—2.5—3	1—2—2.5
	+10 mM NaCl	1—1.2—1.5	1—1.5—2	3—4—4.5	2—2.5—3

The results are expressed as the range of small, mean and large diameter of colonies with the zone of motility on agar covered with semisolid medium containing urea and phenol red. A — fresh agar, B — agar stored for a few days, < — larger motility, I — incubation **of the dishes at 20°C—21°C, II — temperature of incubation 18°C—19°C.**

At room temperature, the decrease rate of motility of *Ps. fluorescens* in the medium with 10 mM NaF was better visible after 5 hours in the fresh media than in the stored medium (Table 1), whereas 10 mM NaCl had not such an effect. The influence of 10 mM $MgCl_2$ revealed itself as the greater zones of motility around the colonies in comparison to control. The *Ps. fluorescens* growing on the agar covered by semisolid medium with phenol red and urea coloured the colonies pink to red after a short time. Proteus changed the medium in a few minutes, also *Salmonella* to yellow with in the zones of motility. Such early colouring of *Pseudomonas fluorescens* was not observed in the seeding tubes with the medium usually using for urease testing microorganisms. In the semisolid medium containing 0.3% of agar it is also possible to test the motility. Activation of motility by $MgCl_2$ was observed in the tubes, turbidity was greater but less coloured, the distance band of motility from meniscus was longer than in the control tubes. Short time was necessary to obtain the colouring of colonies on the dishes covered with semisolid medium, but the colour was more stable in the tubes with the medium containing urea and phenol red even if incubation lasted 48 hours at 37°C. However when 24 hours culture of *Ps. fluorescens* in semisolid agar in the tubes was covered by 3 ml semisolid medium with urea and phenol red, pink colour appeared quickly. Using the method of covering by semisolid medium it is possible to notice the urea positive from urea negative strains and testing the motility at the short time to one an hour. When 10 mM $MgCl_2$ was added to the medium the motility appeared faster even at room temperature. Much smaller zones appeared in the dishes incubated at 18–19°C, than at 20–21°C as is possible to see in Table 1. The motility rate is strongly dependent on temperature.

The findings recorded here show that motility may quickly appear on semi solid agar in the dishes, and that NaF decreased the motility (10 mM) in the semisolid medium, whereas magnesium influenced an increase of motility rate of *Ps. fluorescens*, however the effect depended of environment.

Generally, temperature and other factors as humidity and kind of medium influence the motility appearance.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (559—560)

COOPERATIVITY BETWEEN MYOSIN MOLECULES IN MYOSIN FILAMENTS

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Persechini and Hartshorne [1] observed nonlinear relationship between incorporation of phosphate to gizzard myosin regulatory light chains and actin-activated ATPase activity. They suggest a cooperation between myosin heads, in myosin molecule. In the present paper an evidence for other kind of cooperation in thick filament, dependent on phosphorylation of myosin regulatory light chains is presented. Fully phosphorylated and dephosphorylated myosin was prepared from rabbit back muscle, as described by Michnicka et al. [2]. Preparations were controlled electrophoretically according to Perrie and Perry [3]. Pure actin was prepared as described by Strzelecka-Gołaszewska et al. [4]. Actomyosins were formed from pure actin and phosphorylated and dephosphorylated myosin or mixtures of both.

As shown in Table 1, actin-activited ATPase activities of two forms of myosin are different. The activity of phosphorylated myosin is

TABLE 1

Actin-stimulated Mg^{2+}-ATPase activities of phosphorylated and dephosphorylated myosin; A: 1 mM $MgCl_2$, B: 2 mM $MgCl_2$. ATPase activity was assayed in the medium consisting of 0.1 M KCl, 20 mM Tris-acetate (pH 7.0), 1 mM ATP variable concentrations of $MgCl_2$, 3 mM EGTA, 3 mM $CaCl_2$ or 0.1 mM EGTA. 0.2 mg/ml myosin and 0.067 mg/ml pure actin were added

myosin	ATPase activity in μmoles/mg per min			
	A		B	
	$CaCl_2$	EGTA	$CaCl_2$	EGTA
dephosphorylated	0.065	0.098	0.091	0.095
phosphorylated	0.028	0.061	0.051	0.047

significantly lower in all conditions studied. Fig. 1 shows the effect of mixing of variable amounts of phosphorylated and dephosphorylated myosin on actin-activated ATPase activity. In all cases a nonlinear dependence is established. Similar results were obtained for mixtures of intact myosin and myosin devoid of both regulatory light chains (not

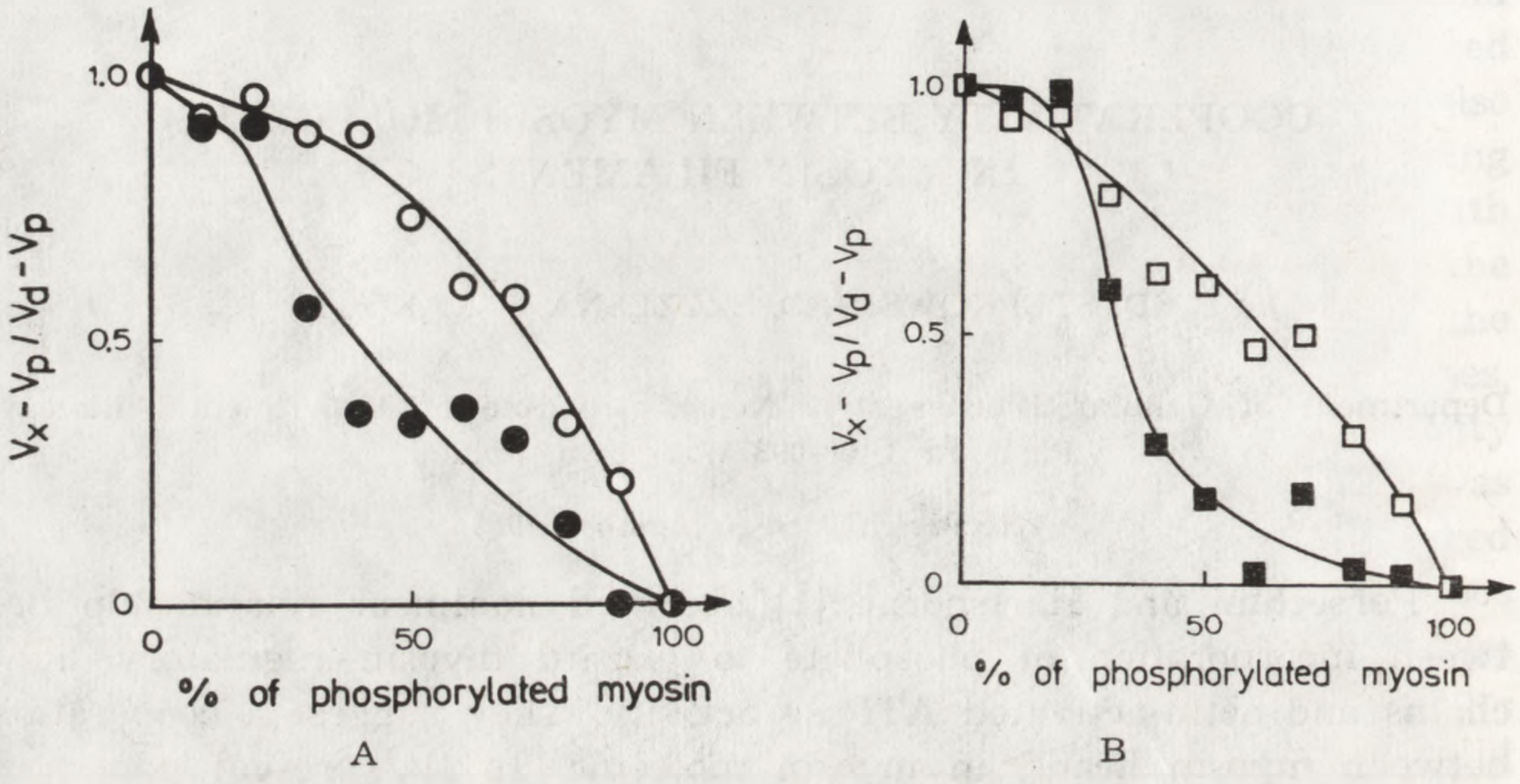

Fig. 1. Correlation between decrease of actin-stimulated Mg^{2+} — ATPase activity of mixture of dephosphorylated and phosphorylated myosin and the amount of the latter. Dephosphorylated myosin was mixed with increased amounts of phosphorylated myosin to that the concentration of protein remained constant. V_d--ATPase activity of dephosphorylated myosin V_p-ATPase activity of phosphorylated myosin V_x-ATPase activity of the appriopriate mixture of both kinds of myosin, 2 mM $MgCl_2$ (●, ■), 1 mM $MgCl_2$ (○, □), *A*: 3 mM $CaCl_2$ and 3 mM EGTA, *B*: 0.1 mM EGTA. In Table 1 respective V_d and V_p values and Mg^{2+}-ATPase conditions are given.

shown). The nonlinear relationship between the amount of phosphorylated myosin and ATPase activity may be caused by the interaction between neighbouring myosin heads in the myosin filament. Therefore the existence of cooperation between heads of different myosin molecules seems to be possible.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (561—564)

THE STABILITY OF FILAMENTS FORMED FROM PHOSPHORYLATED AND DEPHOSPHORYLATED FAST SKELETAL MUSCLE ACTOMYOSIN

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As it has been shown previously the value of specific ATPase activity of natural actomyosin obtained from fast skeletal muscle is significantly decrease when regulatory light chains of myosin are phosphorylated [1]. The phosphorylation of myosin enhanced its affinity for actin and modified actin activated Mg^{2+} ATPase of the enzyme [2, 3]. The latter is rather difficult to interpret because both enhanced affinity of myosin to actin and different stability of phosphorylated and dephosphorylated myosin and actomyosin filaments may contribute to the final results of actomyosin ATPase [1]. As it is demonstrated in the Fig. 1. the increase of the turbidity of actomyosin suspension, showing an in-

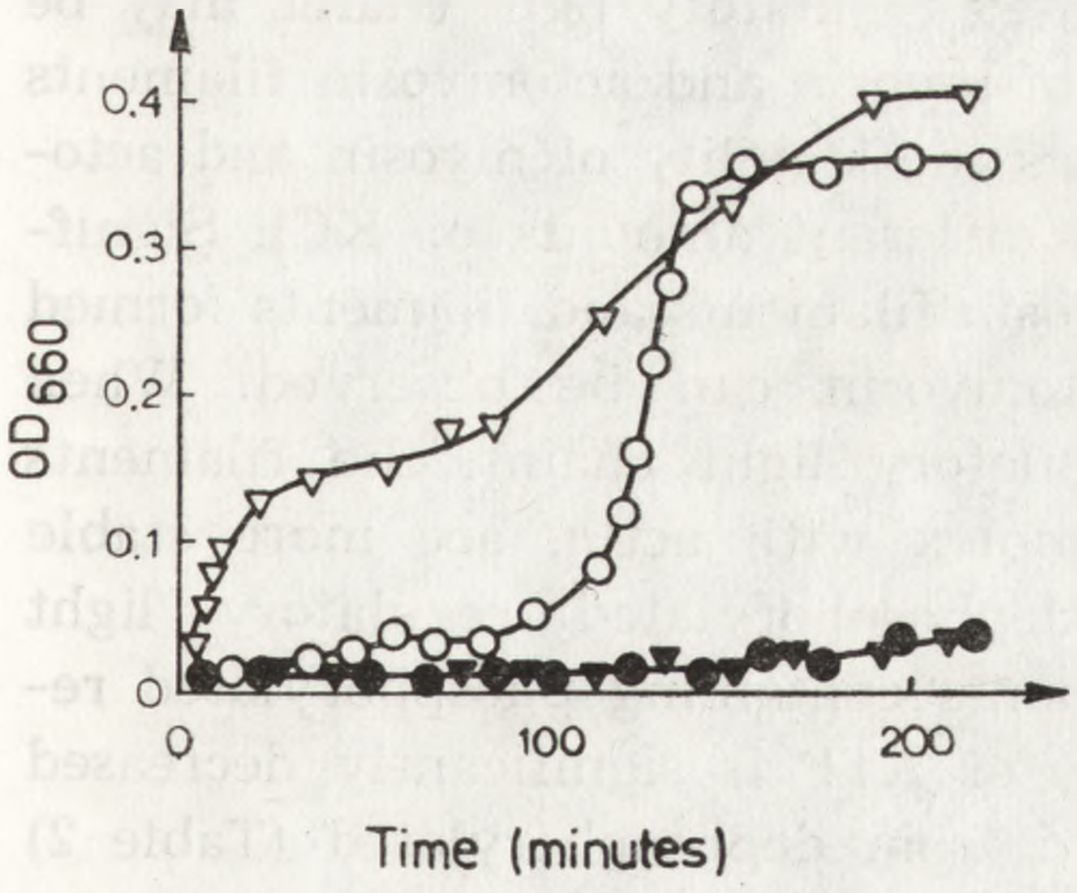

Fig. 1. Superprecipitation of actomyosin suspension containing myosin with phosphorylated (●, ▼) and dephosphorylated (○, ▽) regulatory light chains. The reaction was started by addition of protein to the media containing 0.1 M KCl, 20 mM Tris-acetate (pH 7.0) 1 mM ATP, 3 mM EGTA and 3 mM $CaCl_2$ (●, ○) or 0.1 mM EGTA (▼, ▽). The changes in turbidity were measured at 660 nm wavelegth. Specific ATPase activities of phosphorylated and dephosphorylated myosin were: K^+ ATPase 2.44 and 2.40 μmoles/mg/min, Ca^{2+} ATPase 0.714 and 0.703 μmoles/mg/min actin, stimulated Mg^{2+} ATPase 0.050 and 0.098 μmoles/mg/min, respectively

TABLE 1

Stability of myosin and actomyosin filaments in the presence of variable amounts of KCl. The amount of filaments remaining unsoluble was measured spectrophotometrically 5 min. after addition of KCl and stirring, or calculated from the amount of protein precipitated after 15 min. centrifugation of protein suspension at 20 000 g. The phosphorylated and dephosphorylated myosin and actomyosin were prepared as previously described [1, 2]

Kind of protein used for filament formation	The amount of filament remaining insoluble in % of total amount present in solution containing KCl in concentration of:					
	0.15	0.20	0.25	0.30	0.35	0.40
phosphorylated myosin	95	85	60	50	30	28
dephosphorylated myosin	95	70	52	30	20	10
natural actomyosin	100	100	100	69	55	30
reconstituted phosphorylated actomyosin	100	100	98	65	52	20
reconstituted dephosphorylated actomyosin	100	100	99	60	78	15

creased amount of filaments formed, can be observed few minutes after addition of ATP when actomyosin was formed from actin and myosin containing dephosphorylated regulatory light chains. The rapid mixing of actomyosin containing phosphorylated regulatory light chains with ATP in the same conditions results in dissociation of actomyosin, while the myosin filaments remain dissolved for about two hundred minutes, both in the presence and in the absence of calcium ions. The value of specific K^+ — and Ca^+ ATPase activity of phosphorylated and dephosphorylated myosin used for actomyosin formation were practically identical. The actin-activated ATPase activity of actomyosin determined in the conditions used for the studies of optical density changes (superprecipitation) was lowered when myosin used for actomyosin formation contained phosphorylated light chains. However the lowered ATPase of actomyosin containing phosphorylated regulatory light chains may be a consequence of a changed stability of myosin and actomyosin filaments in the presence of ATP. Table 1 shows the stability of myosin and actomyosin filaments in the presence of different amounts of KCl. Significant difference in the stability of myosin filaments and filaments formed from natural and reconstituted actomyosin can be observed. When myosin contains phosphorylated regulatory light chains, the filaments formed from this myosin or its complex with actin, are more stable compared with those containing dephosphorylated regulatory light chains. The stability of myosin filaments containing phosphorylated regulatory light chains in the presence of ATP is significantly decreased as compared to the filaments formed from dephosphorylated (Table 2)

TABLE 2

Stability of myosin filaments in the presence of variable amounts of MgATP. Myosin filaments were suspended in the solution containing 20 mM Tris-acetate pH 7.0 and 100 mM KCl and variable amounts of ATP. For details see Table 1

Kind of protein used for filament formation	The amount of filaments remaining insoluble in % of total amount present in solution containing MgATP in concentration of:					
	0.5 mM	1.0 mM	1.5 mM	2.0 mM	2.5 mM	3.0 mM
phosphorylated myosin	72	48	39	26	15	2
dephosphorylated myosin	95	82	70	58	52	40

myosin. The findings support the observations made by K. Kasman (unpublished results) who found the differences in the structure and the stability of myosin filaments. Thus the phosphorylation of regulatory light chains may influence the interaction between the myosin molecules forming the synthetic myosin filaments.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (565—568)

THE EFFECT OF TEMPERATURE ON THE MUSCLE RESTING POTENTIAL IN SOME INSECTS *

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Studies on the effects of temperature on excitable tissues have provided important data to the knowledge of the origin of bioelectric potentials. The effect of temperature was investigated in the field of membrane transport and especially active transport. Attention has been also paid to the effect of temperature on the resting potential of neurons and muscle fibres. The latest investigations include the role of temperature in the single channel phenomena. The purpose of the present study was to examine the effect of temperature on the resting potential of the muscle fibres in insects. The experiments were performed on the skeletal muscles of adult forms of *Acheta domesticus* L. and larvae of *Tenebrio molitor* L. (reared in insectaria at room temperature) and adult forms of *Leptinotarsa decemlineata* Say (caught and kept in an insectarium only during the experiments). The conventional microelectrode methods and recording system were used. The resting potential was recorded at room temperature (control experiments), at 4°C and 35°C. A special glass container allowing to keep the temperature at a constant level (4 or 35°C) was used for the experiments. Placing the preparations into the bath at 4°C caused in all three species a rapid decrease of the muscle resting potential (± by 1/3). The increased temperature (±35°C) led usually to a transient, lower or higher, increase of the resting potential (10 min after placing in the solution) and then a decrease of the potential below the control value. Fig. 1 shows the

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results obtained in *Leptinotarsa decemlineata*. The results concerning *Acheta domesticus* and *Tenebrio molitor* were similar. The investigations concerning the effect of temperature on insect and other inver-

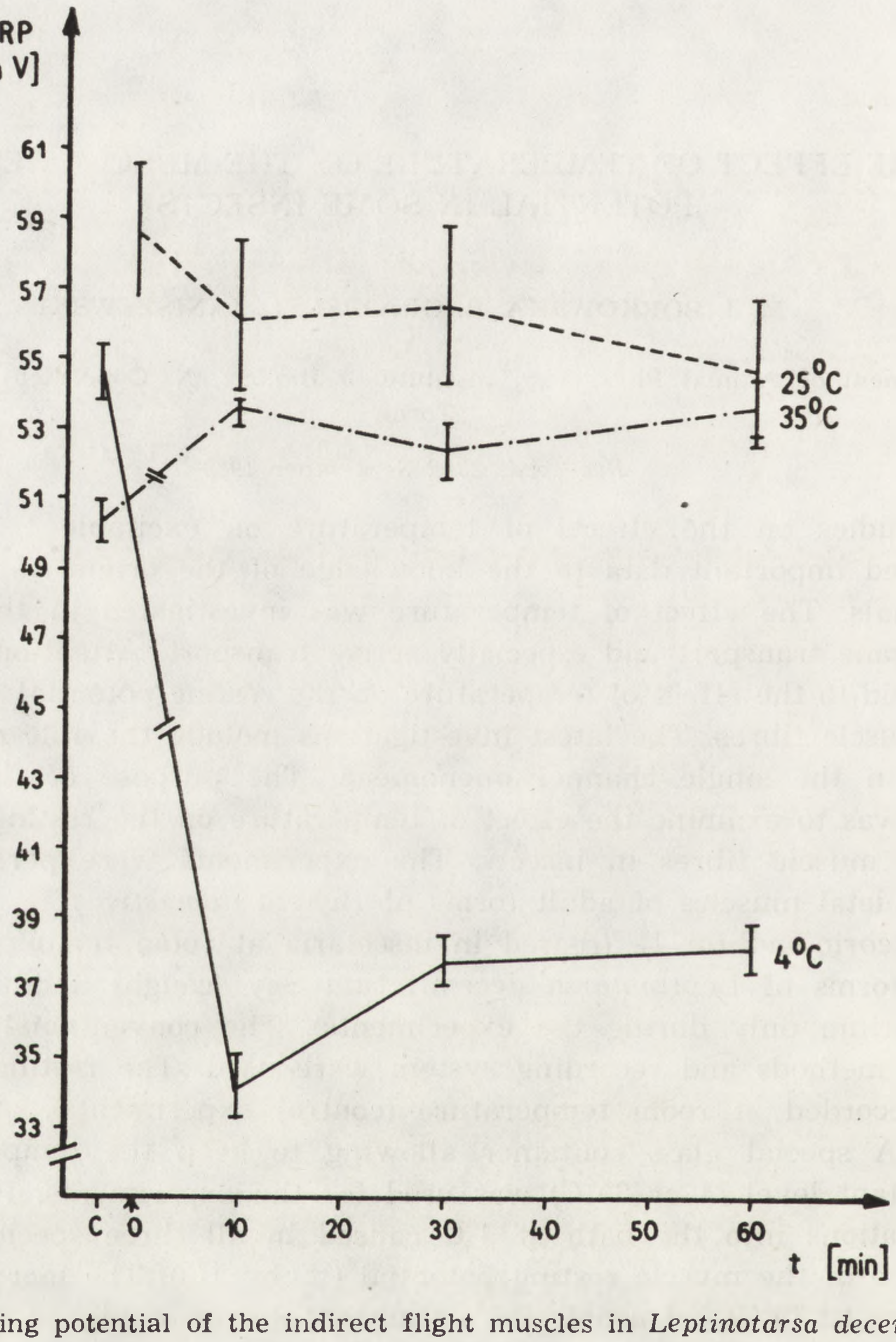

Fig. 1. Resting potential of the indirect flight muscles in *Leptinotarsa decemlineata* measured during 60 min at 4°C, 25°C and 35°C

tebrate muscle fibres came to a general conclusion, that the membrane potential decreases on cooling and increases on warming. This statement had to be expected, according to the Nernst equation. The results of our experiments are in essential agreement with data reported by several

researches. It should be noted that our results showed a marked divergence between the values which could be expected from the Nernst relation and the experimental data. For example in *Leptinotarsa decemlineata* the rapid cooling of the preparation from 25°C to 4°C caused a decrease of muscle resting potential (assuming linear changes) of the order of 1.1 mV/1°C. The theoretical decrease of the membrane potential according to the Nernst equation should be 0.2 mV/1°C. Similar results have been obtained in the mealworm larvae muscle fibres. The cooling from the level of 20°C to 5°C led to a decrease of the membrane potential of 0.9 mV/1°C, whereas the theoretical value taken from the Nernst equation should be equal to 0.1 mV/1°C. These findings may indicate the complex nature of the membrane potential. According to recent views the membrane potential consists of two components: one depends on the ionic gradients and permeabilities in accordance with the Goldman equation, and a second one which depends on the active transport. Recent findings show that temperature may also induce changes of the molecular structure of the membrane. The complex nature of the action of temperature on the muscle resting potential remains still an open question and requires further investigations.

POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (569—572)

NUMBER AND LENGTH OF MICROTUBULES WITHIN ORGANELLES OF NORMAL AND SIZE-REDUCED CELLS OF *DILEPTUS ANSER* (*CILIATA*)

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In this study a diminution of microtubule number and length was found in cortical organelles of size-reduced cells, as compared to normal ones. The organelles studied, were transverse fibres connected with oral ciliature of three different sectors of mouthparts. The cells termed "normal" were taken from growing cultures, and thus were in all stages of cell cycle. Very small fragments were "size-reduced", and fully regenerated 24 hours after transection. Reduction in size caused a three-fold reduction of the cell length (Table 1).

TABLE 1

Cell	Length of trunk	Microtubule number			Number of ribbons
		left fibre	right fibre	cytost. fibre	
normal	319±65 μ m	25.8±2 tubules	15.2±1.1 tubules	16.5±1.9 tubules	12.4±2 ribbons
size-reduced	109±21 μ m	20.6±2.2 tubules	12.2±1.0 tubules	11.9±2.3 tubules	7.0±1 ribbons

Values are given ± S.D.

The cell of *Dileptus* consists of a cylindrical trunk with a slender processus, the so-called proboscis, at its anterior end (Fig. 1*A*). A cytostome is located at the base of the proboscis, and encircled by a non-ciliated cytostomal field. A narrow extension of this field occupies the ventral side of the proboscis. On the circumference of the cytostomal field, together with its extension, a single row of oral ciliature is situ-

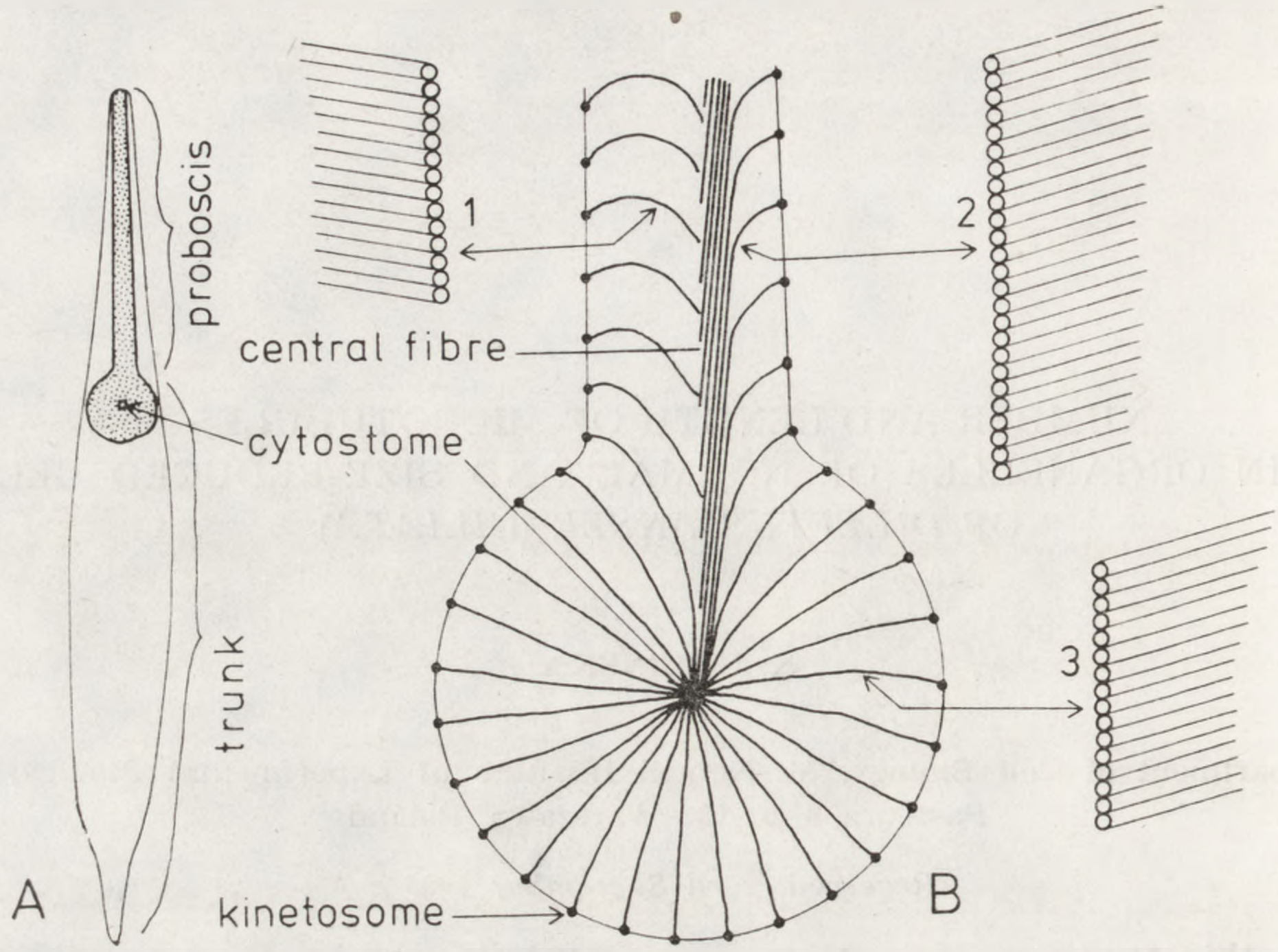

Fig. 1. *A*. Ventral side of *Dileptus*. Dotted area is the cytostomal field. *B*. Pattern of transverse fibres on cytostomal field; *1* — section of right transverse ribbon, *2* — section of left transverse ribbon, *3* — section of transverse ribbon from cytostomal area

ated. Kinetosomes of oral ciliature give their transverse fibres on the territory of the cytostomal field (Fig. 1 *B*). The transverse fibres differ in their microtubule content, according to their location. The largest ribbons are found on the left margin of the extension of the field. Those on the right side are much smaller, and similar in size to those found around the cytostome.

The number of microtubules was estimated in all three kinds of fibres for normal and size-reduced cells. The results are summarized in Table 1. A downward regulation in microtubule numbers was found in size-reduced cells. The three-fold reduction of the length of cells was accompanied by a reduction of microtubule number of about 20%.

The length of microtubules was estimated by counting the left transverse fibres forming a bundle on ventral side of proboscis, the so-called central fibre (Fig. 1 *B*). This bundle is formed by distal parts of several left transverse ribbons, all curved towards the cytostome. Preliminary observations showed that the distances in between kinetosomes of left transverse ribbons are the same in normal and size-reduced cells. So, the number of transverse ribbons on a given section of central fibre allows one to estimate the length of the sectors of ribbons

which enter into the central fibre. A count of the number of ribbons in normal and size-reduced cells, shows that there is a two-fold reduction of microtubule length in size-reduced cells (Table 1). Direct measurement showed that the length of left transverse fibre in normal cell was about 10 μm, in size-reduced cell was about 6 μm.

This study is a continuation of earlier investigations on the morphogenetic possibilities of microtubule-organizing-centres (MTOCs). It has been found that MTOCs connected with kinetosomes may react to factors controlling regulation of cortical domains, by a change in the kind of cilia and rootlets they produce [1], [2]. In this study it appears that the reduction of the overall size of the cell is reflected in newly formed MTOCs of transverse fibres of kinetosomes. The new MTOCs contain a reduced number of microtubule-nucleating sites, since they produce fewer microtubules. The length of microtubules is reduced more severely than their number, leading to a change in internal proportions of transverse fibres. This may indicate that restriction of assembly is controlled on another pathway than the number of nucleating sites in MTOC of *Dileptus*.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (573—574)

BIOELECTRICAL ACTIVITY OF THE PLASMODIUM OF PHYSARUM

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The oscillating electrical activity in the plasmodia of myxomycetes has been studied extensively. Watanabe et al. [10] found that if two glass micropipettes were inserted at any two sites on a plasmodial vein of *Dydinium nogripes,* the potential difference fluctuated rhythmically along its length. Introduction of a simple "double chamber" method to measure cell surface potentials in the slime mold, *Physarum polycephalum,* by Kamiya and Abe [5] initiated numerous electrophysiological investigations on this organism [2, 6]. All the observations, including those with intracellular microelectrode methods [3, 7, 8] provide the existence of rhythmical potential changes in *Physarum* within a few minutes. Using a fluorinert liquid method, Stockem and Meyer [8] showed clearly that electrical oscillations are present in the plasmodium and might be due to "rhythmical ion fluctuations between the slime mold protoplasm and its environment...". This suggestion seems to be true in the light of the recent discovery that protoplasmic free Ca^{+2} and H^{+} ions oscillate in *Physarum* with the same rhythm as contraction-relaxation cycle of plasmodium vein [4, 9]. The present measurements were designed to investigate which ion may be responsible for generating rhythmical changes in the plasmodial potentials of *Physarum polycephalum* recorded externally by microelectrodes. For this purpose a modified fluorinert liquid technique [8] was applied to isolated veins of the plasmodium. The fluorinert liquids FC 43 and FC 77 (3-M Co., USA) were used for electrical isolation of fragmented vein. A pair of microelectrodes, i.e. one glass micropipette filled with 0.5 M NaCl for reference potential and the second, an antimony microelectrode (Transidyne General Co., USA) for sensing H^{+}-activity, were inserted into

a small water droplet deposited at one end of a vein. Both microelectrodes were connected to conventional electronic equipment [1] and continuous monitoring of H^+-activity was performed with a pen recorder. The shuttle streaming of protoplasm was observed by light stereo-microscopy. The H^+-ion activity measurements carried out in fluorinert liquid demonstrated periodic proton level changes in the droplet applied to the fragmented vein. Such oscillating H^+-activity had approximately similar frequence as that observed in the protoplasmic shuttle streaming. Each change in the streaming direction usually coincided with the maximum and minimum values of H^+ level in the water droplet. The study presented here are in progress and will extended, however, the results obtained thus far demonstrate the presence of electrical fluctuations in environment of plasmodial vein and suggest they are caused by proton fluxes across or from membrane in *Physarum.*

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (575—576)

INTRACELLULAR K^+-ACTIVITY AND CELL MEMBRANE POTENTIAL IN *STENTOR*

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It is widely accepted that actual biological phenomena in the living cells are closely related to changes in ionic activity, rather, than to the total chemical concentration changes. A number of measurements on intracellular ion activities in a variety of preparations have been performed with ion-selective microeletrodes by direct puncture of the living cell. In the present study, a K^+-selective, single-barrelled microelectrode was constructed with the use of K^+-exchanger resin (Corning no. 477317, USA) to measure the intracellular free potassium activity in the living protozoan, *Stentor coeruleus*. The technique of measurement was similar to that reported by Lux [2]. The potassium-selective microelectrode system consisted of a pair of micropipettes similar in shape and tip diameter; one for the K^+-selective microelectrode and the other for the membrane potential microelectrode. Both microelec-

TABLE 1

Comparison of values obtained on *Stentor* with intracellular punctures (this report) with those by ultramicroflame photometry [5]

Parameters	This report	[5]
E_m(mV)	-50.8 ± 2.5	-48 to 56
a^i_K(mM)	15.2 ± 0.8	—
c^i_K(mM)	20.3*	12.4
E_K(mV)	-65	—

* Concentration, c^i_K was calculated assuming the cell potassium activity coefficient equals that of external medium (i.e. 0.75; [4]).

trodes were connected to the preamplifiers in the conventional way and electrical responses were recorded with a pen recorder. All measurements were carried out at 12–15°C on *Stentor* cells placed in standard medium containing: 1 mM NaCl, 1.3 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$ and 5 mM Tris-HCl buffer, pH 7.4. The electrical potentials recorded from independent penetrations by conventional and K^+-selective microelectrodes are given in the Table 1. As seen from Table 1, the measured value of cell membrane potential, E_m, is in good agreement with that reported in other studies [1, 3, 5]. The intracellular potassium activity, a^i_K, was found to be between 14.1 mM and 16.4, averaging 15.2 mM. The calculated equilibrium potential for potassium, E_K, from extra- and intracellular ion activities, is considerably higher than that from direct measurement of E_m. This difference between E_m and E_K means that the E_K may be maintained in *Stentor* cells by the permeability of other ions than potassium, or by specific active ion transport. Both mechanisms are known in the cells of higher organisms, while in protozoans further clarification is required. The planned application of other ion-exchager resins for making selective microelectrodes for other ions should provide insight into the mechanism of ionic regulation in protozoan cells.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (577—580)

ULTRASTRUCTURAL AND METABOLIC TRANSFORMATIONS OF THE LARCH MICROSPORE DURING G_1 PERIOD OF THE POST-MEIOTIC INTERPHASE

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The microspore, which constitutes the first step of the plant's haploid phase, is of particular interest for studies on cellular differentiation. On the threshold of the new haploid phase, the nucleus bringing into the young microspore a single set of genes is in the "old" sporophytic cytoplasm, which has previously cooperated with the diploid nucleus. Our studies show that the recovery of the metabolic balance of the system: nucleus-cytoplasm and the laying out of the new development line leading to gametogenesis, are reached through intensive structural and metabolic transformations of the microspore. These transformations take place during the comparatively long post-meiotic interphase. In *Larix europaea*, the post-meiotic interphase takes 10–12 days under natural conditions. The longest period falls on phase G_1. Autoradiographic studies with the application of H^3 thymidine have revealed that nuclear DNA replication occurs towards the end of the interphase, and is linked with the easily distinguishable morphological characteristics of the miscrospore. These developmental properties of the larch microspore and the possibility of obtaining microspore fractions representing the successive interphase steps by differential centrifugation in a non-linear density gradient of succrose, have been used for complex studies on the post-meiotic interphase of microspores. The autoradiographic and biochemical methods, as well as standard electron microscopy methods, have revealed that in microspores in phase G_1 rapid metabolic events accompanied by equally striking structural changes

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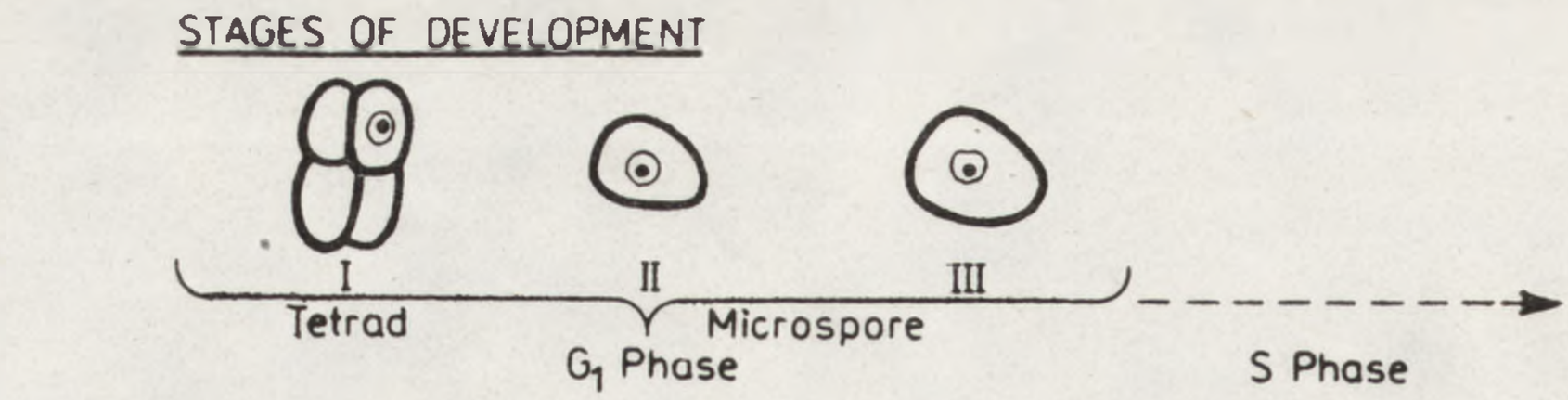

Fig. 1. Ultrastructural and metabolic transformations of larch microspore during G_1 period of the post-meiotic interphase

Variation range of determinants of metabolic activity of microspores: Mg^{+2}-ATPase — 0.75–6.1 [μmol Pi/30 min. × 1cell] × 10^6; cytochrome c-oxidase-0.75–4.1 [μmol ox. cyt. c./min. × × 1 cell] × 10^4; acid phosphatase-2.17-9.0 [μmol Pi/30 min. × 1 cell] × 10^6; LD Pase-0.76-3.6 [μmol Pi/30 min. × 1 cell] × 10^3; peroxidase-1.3-3.78 [K'/1 cell] × 10^5; catalase-0.2-1.2 [nmol O_2/min. × 1 cell] × 10^7; α and β amylase-0.25-0.4 [a.u./1 cell] × 10^7; IAA-1.1-5.5 [mg IAA/1 cell] × 10^7; protein-0.8-20.0 [mg/1 cell] × 10^6; incorporation of ^{14}C-leucine-1.0-22.2 [c.p.m./1 cell] × 10^6; incorporation of ^{3}H-uridine-0-8.2 [number of traces/26 μm^2].

take place (Fig. 1). Here belong: 1. A rise in the level of endogenous auxin (IAA) with which is correlated transitional drop in peroxidase activity [1], 2. a resumption of RNA synthesis after a period of its complete inhibition lasting from the diplotene, 3. intensive protein synthesis resulting in a rapid and considerable increase in total protein content. Electrophoretic separation of the protein fraction demonstrates the appearance of a new protein which did not occur in earlier stages of microsporogenesis [2], 4. an increase in the activities of Mg^{+} ATPase, cytochrome c-oxidase, inozine diphosphatase, acid phosphatase and catalase, 5. a drop in α and β-amylase activity. All these rapid metabolic events are associated with a considerable increase in cell volume and intensive structural transformations in both the nucleus and the cytoplasmic organelles. In the nucleus of the young microspore, a temporary appearance of numerous extranucleolar ribonucleoprotein bodies, which are the structural expression of the new genetic activity of the nucleus was noted [3]. This process in the tetrad stage is associated with phenomena which seem to point to enhanced nucleo-cytoplasmic interaction. Here belong, in the first place, the conspicuous evaginations of the external membrane of the nuclear envelope appearing at that time, and associated with the formation of cytoplasmic vacuoles, as well as the development of nuclear vacuoles. The most striking transformations of cytoplasmic organelles concern the mitochondria and dictyosomes. The mitochondria exhibit a progressing development of the inner membrane, while the dictyosomes undergo rapid transformation from inactive structures in the tetrad stage, into active structures producing numerous vesicles. Moreover, in the time from the tetrad stage to the freed microspore stage, a considerable increase in the number of dictyosomes and amyloplasts was found. The character of the quantitative and ultrastructural changes noted in the population of mitochondria and dictyosomes, proved to be in agreement with the results of biochemical studies, which pointed to an increase in the activity of marker enzymes of these organelles: cytochrome c-oxidase and inosinodiphosphatase. The results of the present study indicate that the threshold of the haploid phase in the development of the male gametophyte, constitutes an important metabolic switch directing the cell on to a new line of development.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (581—584)

METABOLIC ACTIVITY OF CELLS UNDERGOING ANTHER MEIOSIS IN *LARIX EUROPAEA* D.C.

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The work was aimed at a study of the metabolic activity of microsporocytes in successive stages of meiosis in higher plants.

The material for study was fractions of microsporocytes in the same stages of development, and somatic cells obtained from anthers of the larch (*Larix europaea* D.C.) [1]. The study covered twenty-one developmental stages of meiosis, and five stages of the haploid phase. Tests were taken for protein content and for protein synthesis intensity. Also, electrophoretic separation of proteins and isoenzymes of acid phosphatase was carried out, as well as tests for activity of eight enzymes. Most attention was given to enzymes regarded as markers for certain cellular structures or metabolic processes, viz. cytochrome c-oxidase, IDPase, catalase, RNase, DNase, Mg^{+2} — ATPase, α and β amylase, acid phosphatase.

A number of metbolic correlations were observed both within the developing microsporocytes and between microsporocytes and the anther somatic cells. It has been demonstrated that the dynamics and character of metabolic activity variations of microsporocytes and of the anther somatic cells, are clearly different. It has also been found that the microsporocytes showed a metabolic dissimilarity at nearly every meiosis stage studied. A number of data have been obtained demonstrating a clear difference in metabolic activity of the generative line cells in the diplo- and haploid phase.

The variability degree of the protein pattern points to more intensive differentiation processes in cells undergoing meiosis, than in the cells of the anther wall [2]. An analysis of the changes of the electropho-

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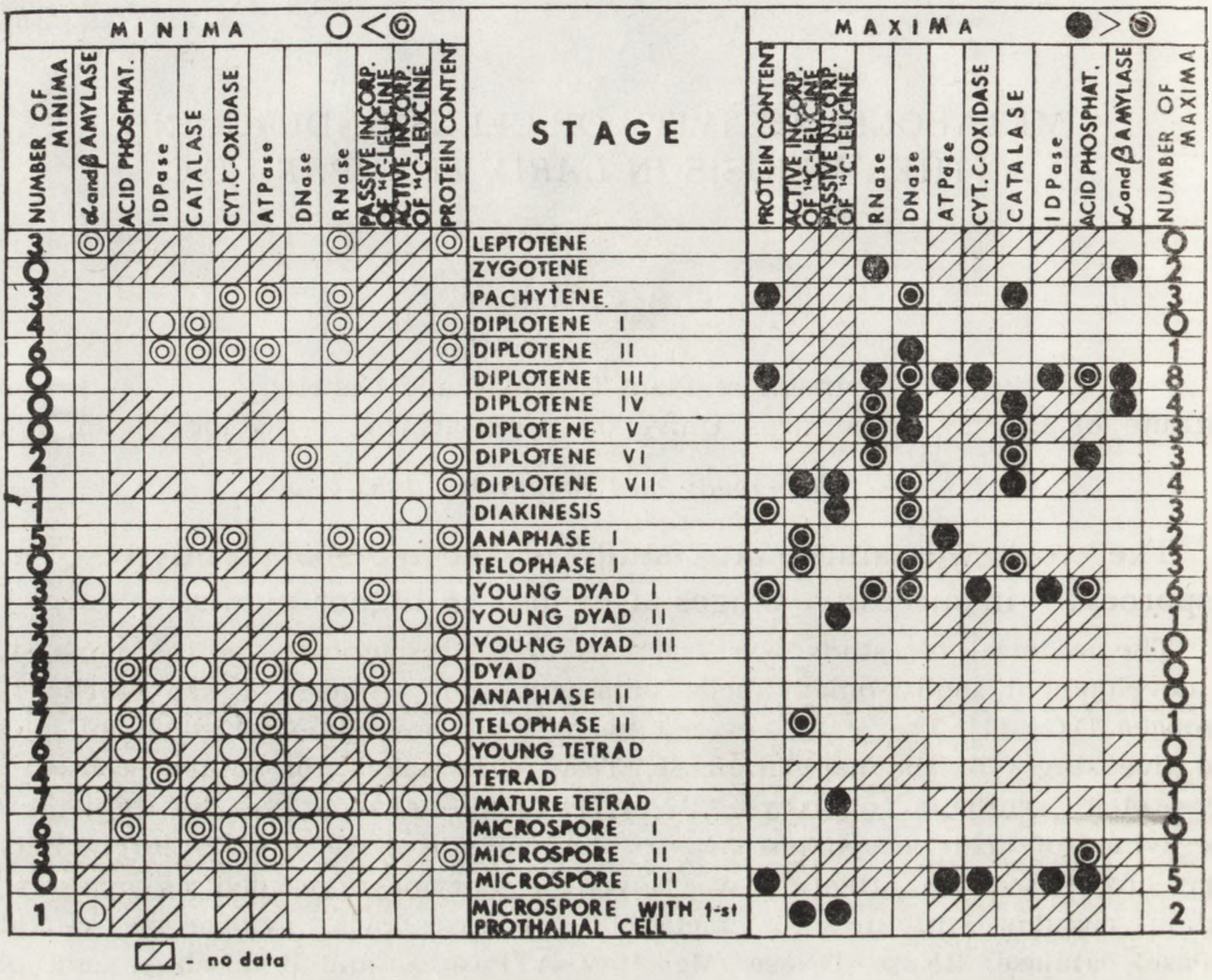
TABLE 1

Occurrence of maximum and minimum values of metabolic activity determinants of microsporocytes during microsporogenesis in larch (all results normalized to one cell, dyad and tetrad are treated as consisting of two and four cells, respectively)



retic pattern of protein fractions [2], acid phosphatase [prepared for publication] and peroxidase [3] isoenzymes, allows us to formulate the hypothesis that the process of microsporocyte differentiation is associated rather with the elimination of certain proteins from metabolism, than with the introduction of new ones. Throughout microsporogenesis a slight decrease in metabolic activity in comparison with the anther somatic cells is noted. Only processes with the participation of RNase, DNase and α and β amylase occur in the microsporocytes throughout meiosis with much lower intensity, compared with the anther somatic cells [prepared for publication]. It is worth noting that the dynamics of variations in the level of enzymatic activities during microsporogenesis is high, in comparison with the anther somatic cells. A striking characteristic of microsporocytes compared with the anther somatic cells, is the greater intensity of transformations of mitochondria, whose determinant is the activity of cytochrome c-oxidase. Periods of increased and

decreased metabolic activity of microsporocytes and somatic cells, do not coincide with the same stages of microsporogenesis. For microsporocytes, the following are the periods of increased activity: the young dyad stage, the middle period of diplotene and the microspore stage before the first mitotic division, whereas increased activity of the anther wall cells was observed in periods corresponding to the diakinesis of microsporocytes, and to the dyad stage. The well-known principle of interaction between the nucleus and the cytoplasm, authorizes the presumption that all the transformations of structure undergone by the nucleus during meiosis are accompanied by continuous restructuring of cytoplasm [4]. This is confirmed by the present results, which reveal violent variations in the activities of marker enzymes of such organelles as mitochondria, the Golgi apparatus and microbodies. On the grounds of these results, it is possible to say that both the first and the second meiotic division are associated with intensive transformations of cytoplasmic structures, but they are particularly striking in the middle diplotene and the young dyad stage. On the verge of the haploid phase, in tetrads, an increase in marker enzyme activity of the above-mentioned organelles has also been observed, but not so high as in the young dyad stage. It can therefore be assumed that the restructuring of the cytoplasm supplying the haploid nucleus, starts long before such nucleus forms, viz. in the young dyad stage.

The results of the experiments have revealed that the widely accepted division of meiosis, based on criteria of morphological changes within the nuclear apparatus, does not reflect important and metabolically distinct developmental stages of cells undergoing meiosis. The metabolism of microsporocytes not infrequently undergoes important transformations in a much shorter time than indicated by the still accepted meiosis stages. It has been found that particularly the young dyad and diplotene stages include metabolically very different phases. It is suggested that additional, or different from those so far accepted, stages of meiosis should be introduced, which would take into account the differences in the metabolic state of cells in the particular moments of meiosis.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (585—585)

THE DIFFERENTIATION OF POLLEN CELLS IN *HYACINTHUS ORIENTALIS* L. — ULTRASTRUCTURAL AND METABOLIC STUDIES

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The development of a bicellular pollen grain provides a model situation for studies on cellular differentiation, because proceeding in a nearly closed system formed by the thick sporoderm layer, it leads to a functional differentiation of the pollen sister cells. The generative cell, after dividing into two sperm cells, will perform reproductive functions, while the vegetative cell will grow into the pollen-tube.

In search of the mechanisms determining the divergence of the development lines of pollen cells, a close study was carried out of the metabolic and ultrastructural transformations these cells undergo in the course of the entire maturation process of the pollen grain.

The results of the experiments have revealed that the pollen cells differ from each other in the occurrence of the DNA synthesis period. In both of them, DNA replication takes place after the disappearance of the callose wall, but in the generative cell it starts and ends earlier than in the vegetative cell. The differences also concern the kind of histones and the duration of their synthesis.

The RNA and protein synthesis analyzed against the interphase periods of pollen cells and their growth dynamics, allows to forward the hypothesis that in the vegetative cell the RNA and protein synthesis is used in the first place for the growth of the cell. In the non-growing generative cell, the RNA and protein synthesis is linked chiefly with the preparation of the genetic apparatus for gametes formation.

Research supported in part by a grant from Polish Academy of Sciences and MNSzWiT (MR) II. 2 (B.2) 111B.

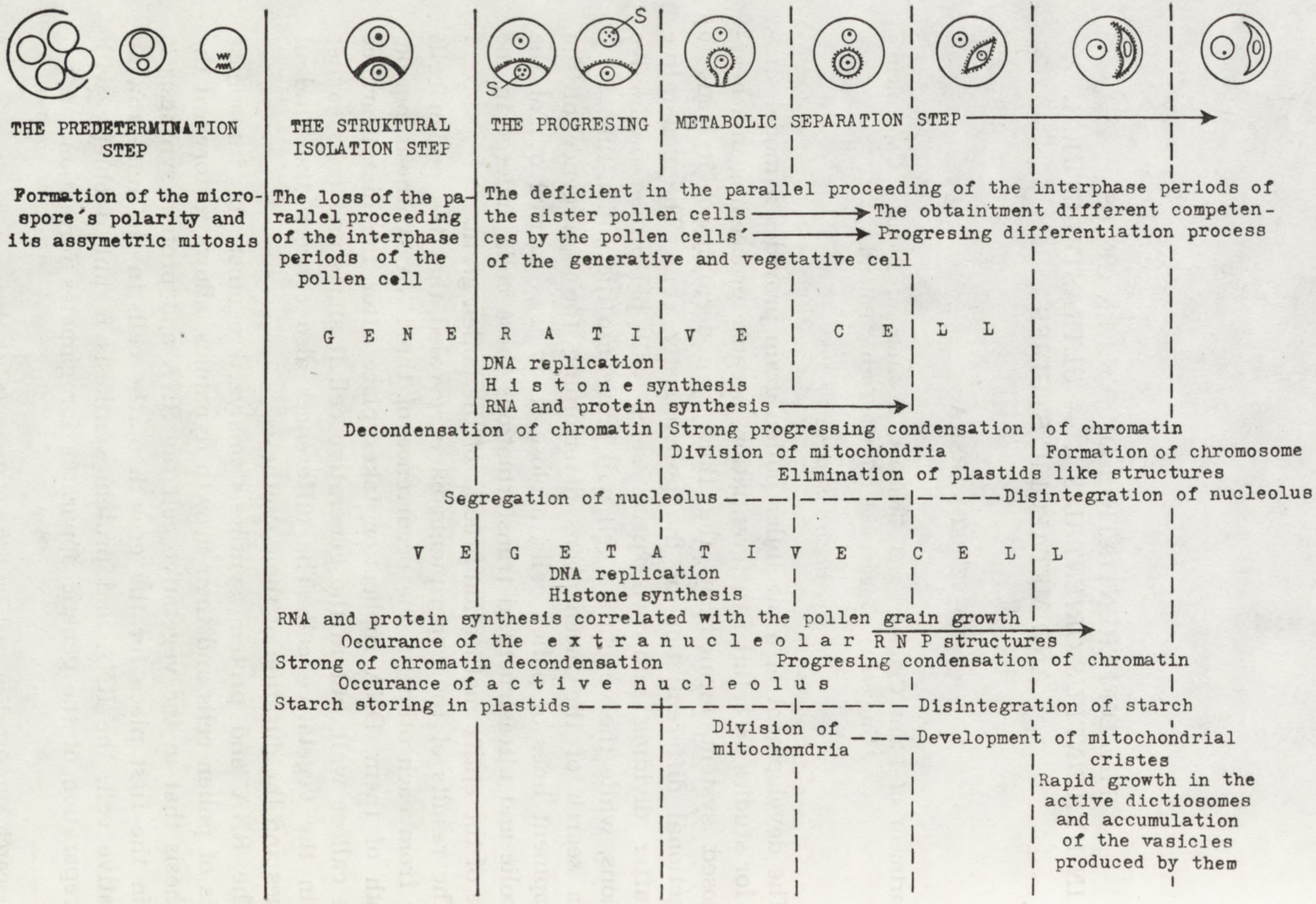

Fig. 1. The main steps of divergence of the developments of the pollen cells

The different metabolisms of the generative and the vegetative cell are associated with different ultrastructural transformations in each of them. The ultrastructural transformations of the generative cell reflect the preparation of its nuclear and cytoplasmic genetic material to be transmitted to the descendant organism. In the generative nucleus, after DNA replication, there occur processes conducive to its entering the early division period. These are: progressing chromatin condensation, the development of prophase chromosome-like structures, the disappearance of the nucleolus preceded by its segregation. In the cytoplasm, there occurs an increase in the number of mitochondria which are present in the mature generative cell, and the elimination of plastids.

The ultrastructural transformations of the vegetative cell reflect the preparation of its substrate and energy potential to the growth of the pollen tube. It includes the development line of plastids which store starch, undergoing disintegration shortly before anthesis, an increase in the number of mitochondria, whose cristae undergo intensive development in the end stage of maturation of the pollen grain, a rapid growth of the number of dictyosomes and the accumulation of vesicles produced by the latter in the cytoplasm of the mature vegetative cell.

Basing on the results of the present experiments, as well as on data from literature, the opinion has been formulated that the determination of the divergence of the developmental lines of pollen cells goes through three main steps: (Fig. 1) 1 — predetermination, 2 — structural isolation, 3 — progressing metabolic separation. The predetermination step covers the time from the tetrad to the microspore division, and manifests itself by the formation of the microspore's polarity into the generative pole and the vegetative pole. The polarized microspore enters asymmetric mitosis, which results in the formation of the generative cell and the vegetative cell. The structural isolation step corresponds to the time of separation of the pollen cells by a callose wall. In that developmental step, the parallel proceeding of their interphase periods is lost. The progressing metabolic separation step lasts from the disappearance of the callose wall, to anthesis. The pollen cells, each in a different stage of its interphase, acquire different competences, and need not be separated structurally. Thanks to metabolic separation being within reach of the same endogenous stimuli, the sister cells can follow different development programs leading to their functional differentiation.

POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (589—592)

VARIOUS PATHWAYS OF SISTER CELLS DIFFERENTIATION, VEGETATIVE AND GENERATIVE, IN THE POLLEN GRAIN OF *TRADESCANTIA*

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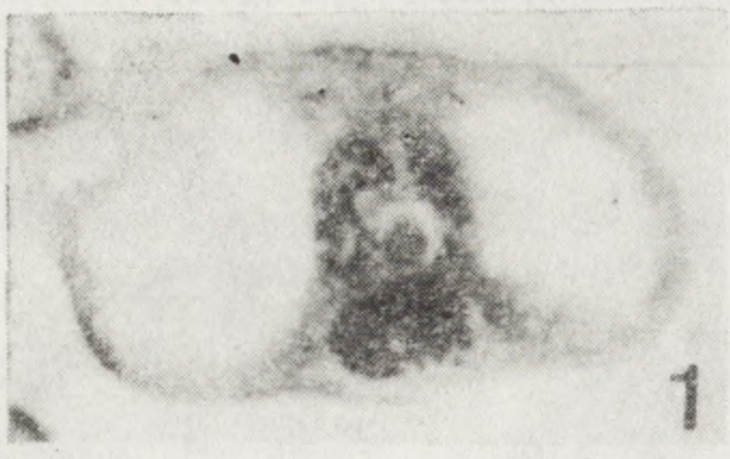
The vegetative and generative cells of *Tradescantia bracteata*, which differ in the size, shape, and organells they contain, and in their destination, are formed similarly as in all other Angiosperms, by polar microspore division, and are surrounded by a common pollen wall. The vegetative cell forms the pollen tube, the generative cell divides to from two sperm cells: Each of the sister cells differentiates in a distinct way, which is why the pollen grain is a convenient object for studies on the molecular mechanisms and ultrastructural aspects of this process [3, 6].

Mature pollen grain of *Tradescantia bracteata* is two-celled. Its development (from first haploid mitosis to anthesis) lasts for six days [2]. The generative cell, which is located by the sporoderm and highly vacuolized bigger vegetative cell is separated by a callose wall [4], and after it dissolution, after the generative cell translocates into the vegetative cell, by a periplasmalemmatic space.

Differentiation of the two cells in the pollen grain of *Tradescantia* takes place in different phases of the cell cycle. DNA synthesis was detected by autoradiografic methods using ^3H thymidine in the generative cell nucleus only [2]. After the short period of the G_1 and S phases ($G_1+S=24h$), the generative cell differentiates in the G_2 phase which lasts for five days. The main cytological aspects of the differentiation are change of the generative cell and its nucleus shapes from the spherical to the spindle-like, gradual chromatin condensation, and development of the cytoplasmatic microtubules, which plays the role of the cytoskeleton (Figs. 1–3 and 5). The generative cell contains mitochondria, few



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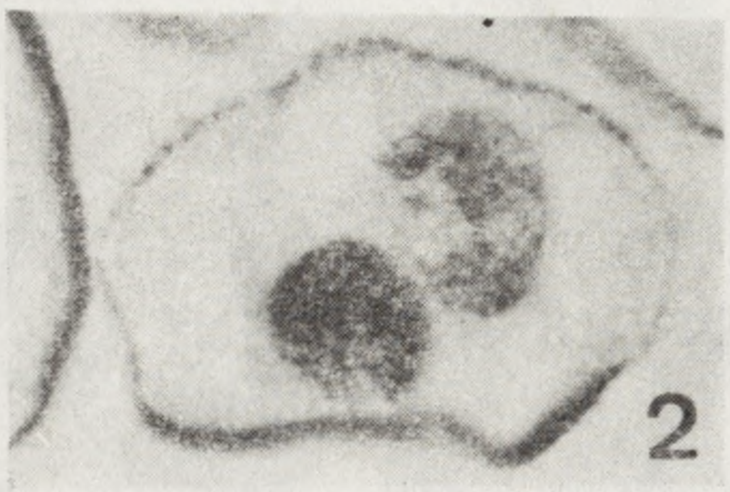

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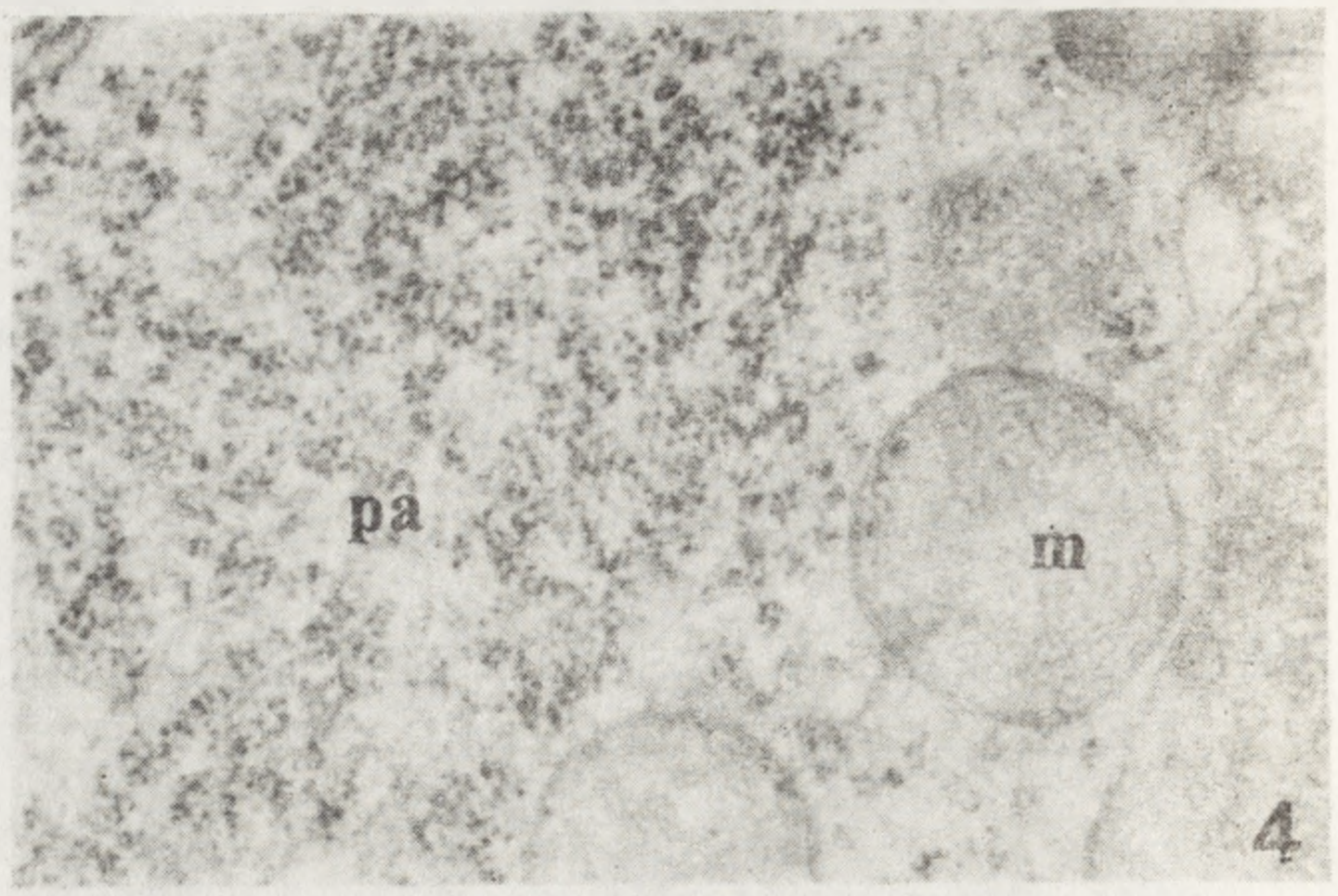

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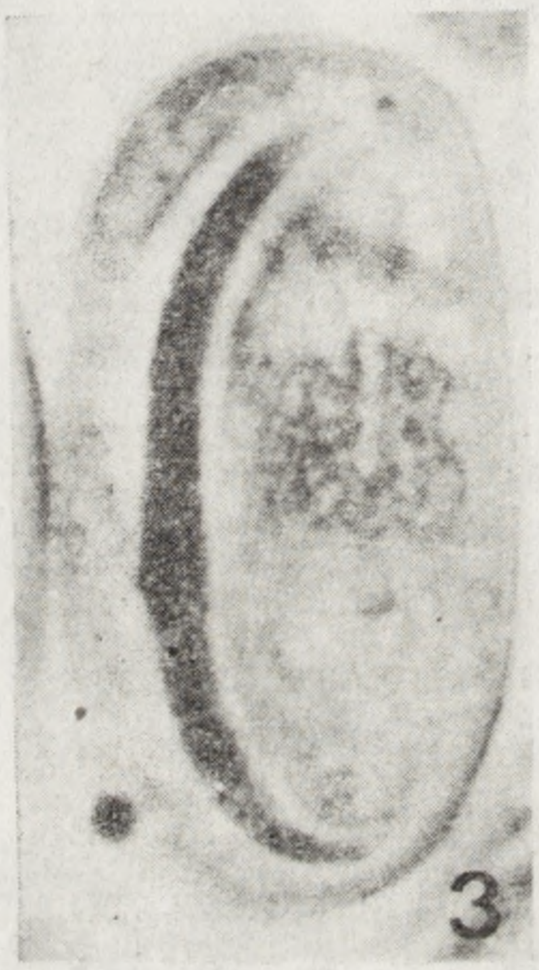

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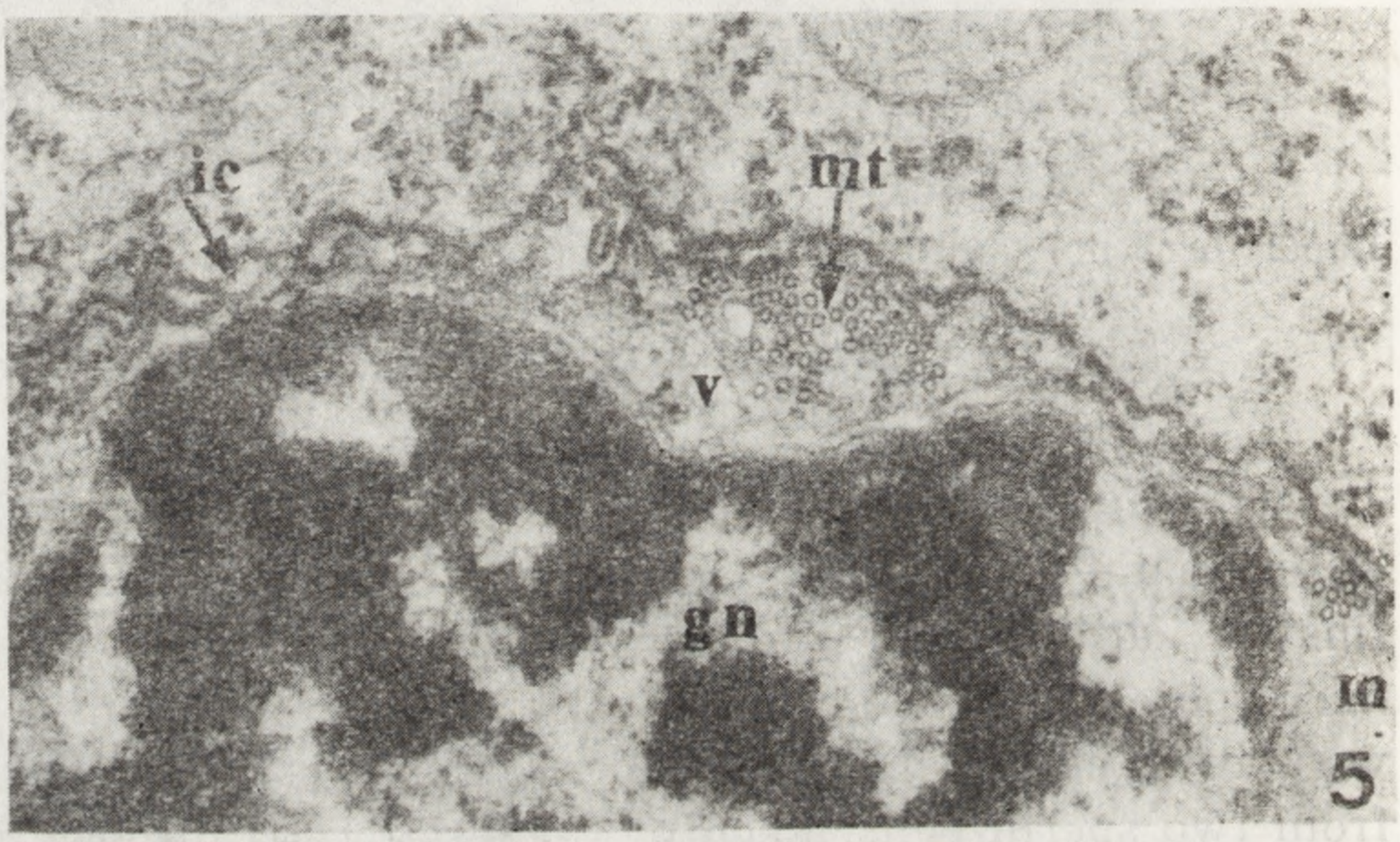

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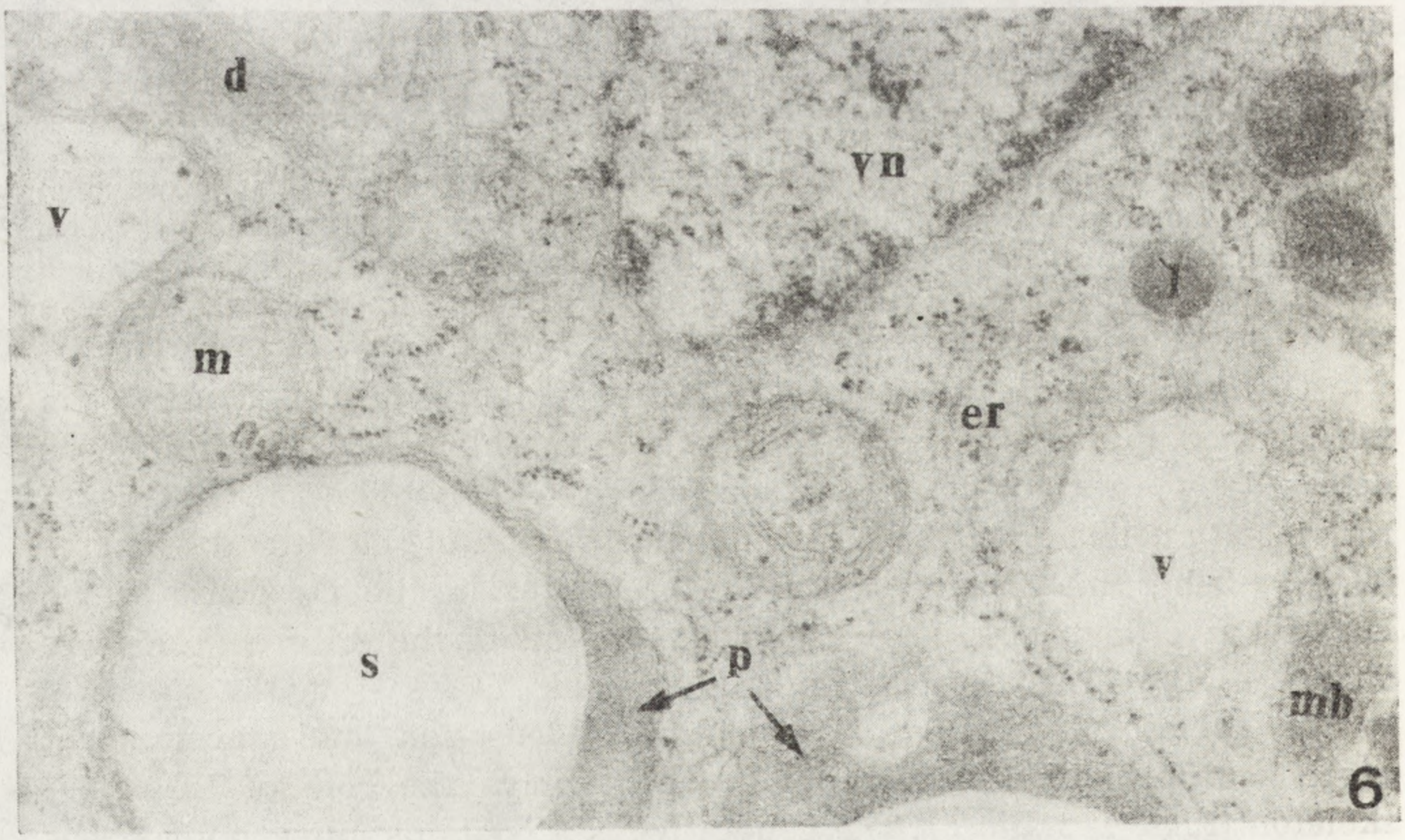

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dictyosomes, weakly developed ER, ribosomes and small vacuoles. Plastids are not present, lipid bodies and microbodies have not been found.

In the vegetative cell nucleus of *Tradescantia*, DNA synthesis has not been found [2]. Therefore, the vegetative cell differentiates in the G_1 phase which lasts for six days. A large increase in the volume and surface of the vegetative nucleus, numerous pores in the nuclear envelope, decondensation of chromatin and large nucleolus, provides evidence of the transcriptive activity of the vegetative nucleus in the postmitotic period. The vegetative nucleus does not contain nucleolus, and its chromatin is more condensed during the late period of the pollen grain maturation. During growth and maturation of the vegetative cell, the vacuoles progressively diminish in size. During this period, the vegetative cell contains numerous mitochondria, dictyosomes, few microbodies, rough ER and characteristic polyribosome areas (Figs. 4 and 6). Vegetative cell devacuolation is accompanied by starch accumulation in the plastids. The day before shedding, plastid starch is decreased, and many lipid bodies appear in cytoplasm. Rapid starch decrease takes place during a few hours in the night, before the anthesis and mature vegetative cell is starchless.

The ultrastructural results found in this work, which indicate the metabolic activity of the vegetative cell nucleus in the postmitotic period, and lack of such activity in the maturating pollen grain, agree with the biochemical data on the RNA-s synthesis [6] and increase of the non-histone protein content [7] during the same stage of development. The lack of DNA synthesis in the vegetative nucleus of *Tradescantia* was determined by cytophotometric study by Thiebaud and Ruch [7], but because of evidence on DNA synthesis in vegetative nuclei in other species [1], it cannot be considered as an essential reason for distinct differentiations of the two pollen grain cells.

Figs. 1-3 Two-celled pollen grain of *Tradescantia bracteata*: after microspore mitosis (Fig. 1), during vegetative cell devacuolation (Fig. 2), and the day before anthesis (Fig. 3). Light microscopy, CrAF fixation, basic fuchsin stained, ca × 1000

Fig. 4. An area of polirybosomes in vegetative cytoplasm from postmitotic phase (day 2 of two-celled pollen grain development), × 36 000

Fig. 5. Cross-section through the portion of elongated generative cell (day 4 of pollen grain development), × 48 000

Fig. 6. Parts of vegetative cell of the same pollen grain as in fig. 5, × 36 000

Figs. 4-6. Electron microscopy, glutaraldehyde and osmium tetroxide fixation, uranyl acetate and lead citrate stained

Abbreviations used: d — dictyosome, er — endoplasmic reticulum, gn — generative nucleus, ic — intercellular space, l — lipid body, m — mitochondrion, mb — microbody, mt — microtubules, p — plastids, pa — polirybosome area, s — starch grain, v — vacuole, vn — vegetative nucleus

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (593—596)

THE ULTRASTRUCTURE OF THE RYE PROEMBRYO

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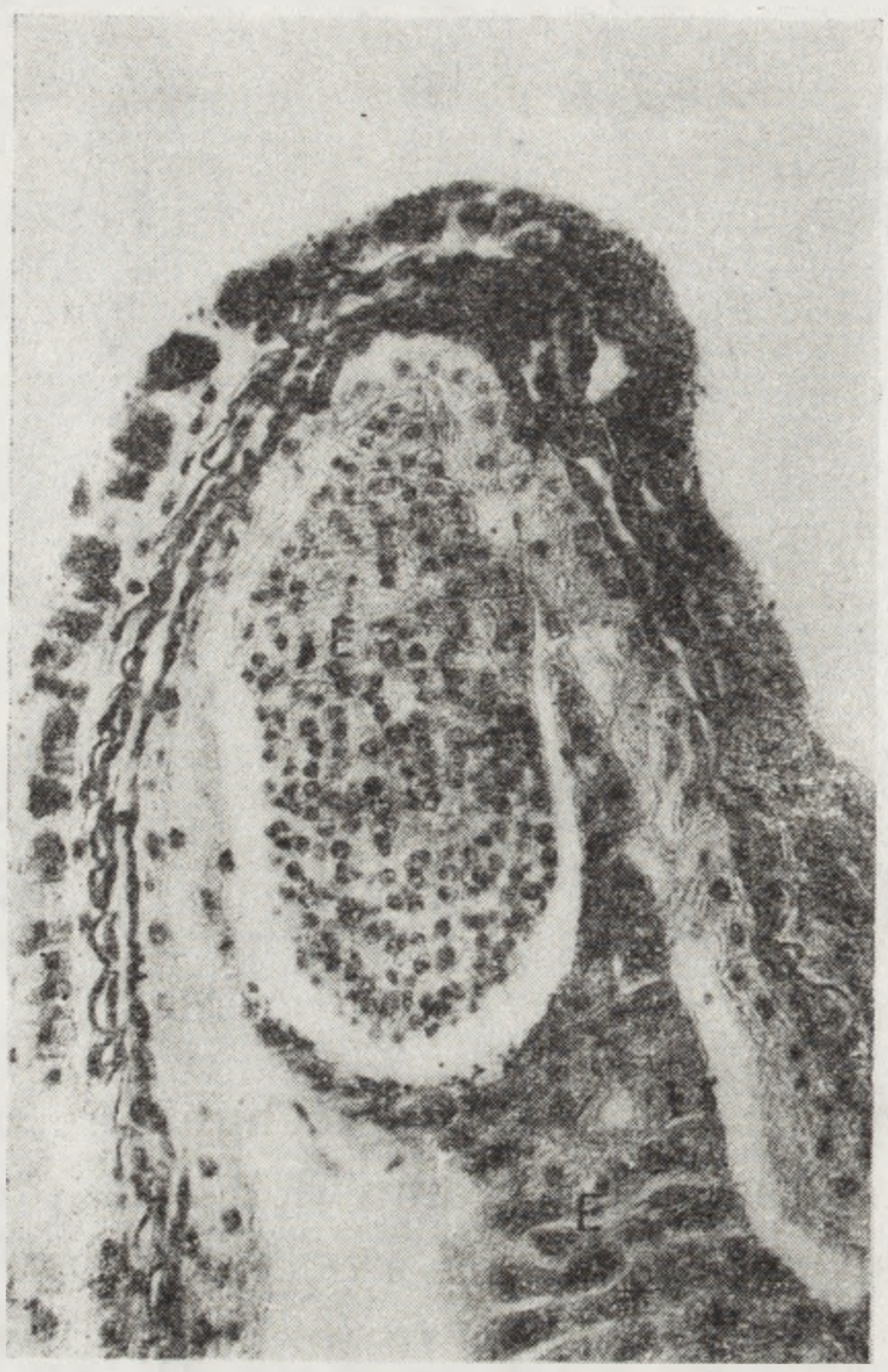
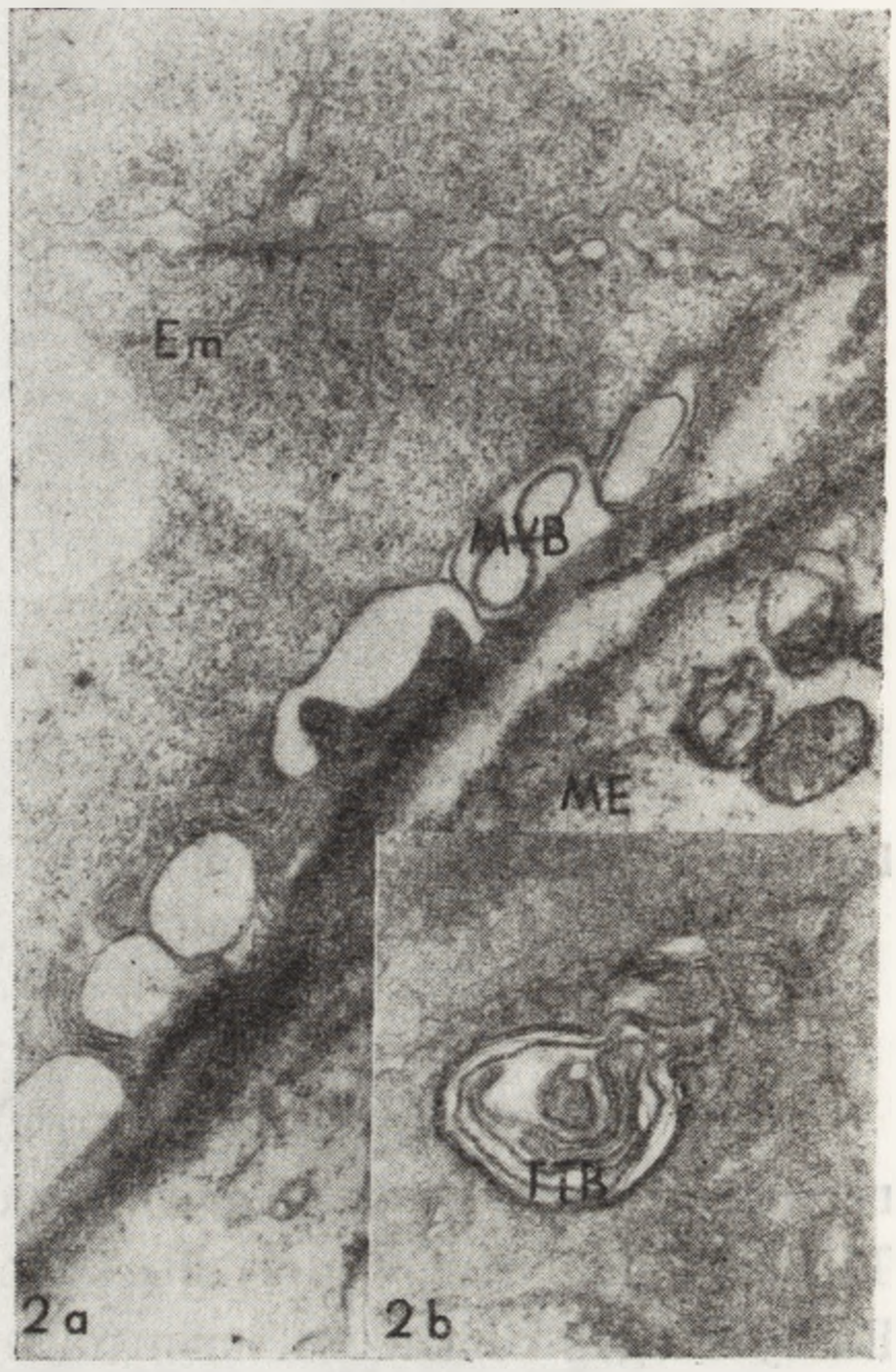
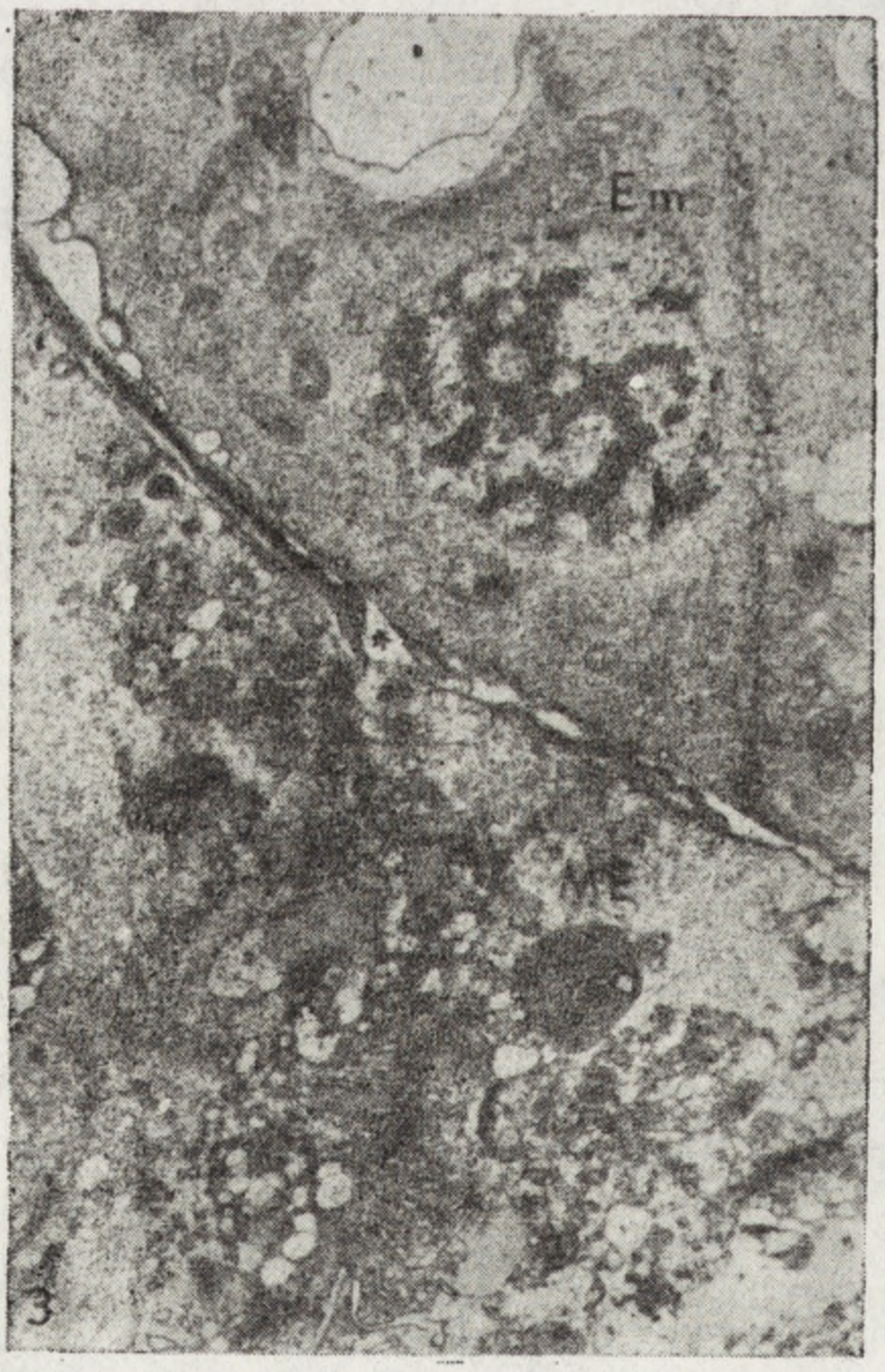
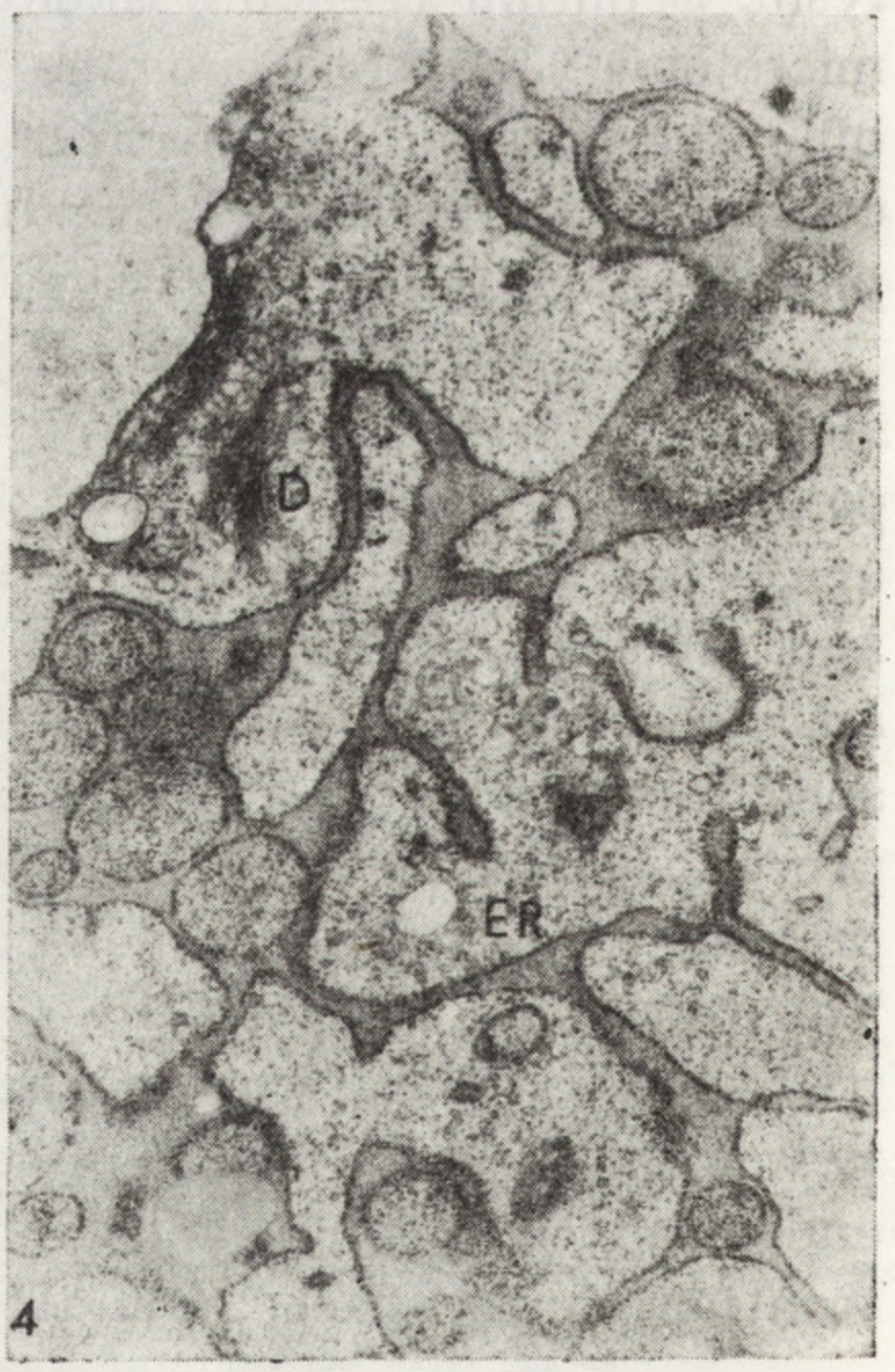
The development of cereal axial organs and the mechanisms of receiving nutrient by proembryo have not been studied in detail before. The present study describes the ultrastructure of the rye proembryo. Early stages of the differentiating cells, and the structure (or structures) participating in the transfer of the nutrient from the endosperm to the proembryo, were investigated. Proembryos of rye, *Secale cereale* L. cv. "Strzekęcińskie" after 7 days of pollination, were excised. Then, they were fixed, dehydrated with standard methods, and embedded in Spurr's resin. Semithin (1–4 μm) and ultrathin sections were cut on an ultramicrotome LKB — "Ultrotome III". Sections were stained with toluidine blue for observations in a light microscope. Ultrathin sections, after contrasting with uranyl acetate and lead citrate, were observed and photographed in a transmission electron microscope.

The oval (long axis ca. 150 μm) 7-day proembryo consisted of meristematic cells (Fig. 1). A high concentration of the numerous vauoles containing multivesicular and tubular-type bodies, was noticed in the apical part of the prombryo also contained plasmatubules-like structures and tubular-type bodies bound to cells walls (Fig. 2). The cells of the apical part of the prombryo also contained plasmatubules-like structures specific to apoplast — symplast areas. Around the apical part of the proembryo, a modified endosperm region has been observed. In rye proembryo, two types of cells of modified endosperm have been shown. The cells of the first type (they were in the region adjacent to the aplical part of proembryo) were degenerated — Fig. 3. The cells of the second type were characterized by electron-dense cytoplasm, large quantities of endoplasmic reticulum and diktyosomes (Fig. 4). A relatively wide electrontranslucent space around the whole proembryo has been observed,









From the present ultrastructural study of the apical rye proembryo cells and of the modified endosperm cells, together with the results of the other authors, we can conclude the following: (a) early stages of scutellum differentiation, (b) high solute flux from endosperm to the proembryo.

Fig. 1. Longitudinal section of the rye proembryo 7 days after pollination. Em — embryo, E — endosperm, × 400

Fig. 2. Multivesicular and tubular-type bodies in the apical cells of the proembryo. Em — embryo, ME — modified endosperm, MVB — multivesicular body, TTB — tubular-type body; (*a*) × 22 000, (*b*) × 16 000

Fig. 3. Apical part of the proembryo and the first type, degenerated cells of the modified endosperm. Em — embryo, ME — modified endosperm, asterisk — electron-translucent space, × 9 000

Fig. 4. The second type cells of the modified endosperm. D — diktyosome, ER — endoplasmic reticulum, × 24 000

POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (597—598)

CYTOPLASMIC CONTROL OF CHROMOSOME CONDENSATION IN MOUSE MEIOTIC AND MITOTIC CELLS

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Hybrid cells were produced with the help of Sendai virus in order to study the nucleo-cytoplasmic interactions in mouse meiotic and mitotic cells. Fully grown maturing oocytes (and their anucleate fragments) or mature secondary oocytes, small immature oocytes and blastomeres were fused together in different combinations. From the results, the following conclusions were made:

1. The process of maturation of mouse oocytes in controlled by a cytoplasmic factor which induces breakdown of nuclear membrane and chromosome condensation. Fully grown oocytes contain or produce this factor.

2. In small immature oocytes, the cytoplasmic factor is either absent or present in an insufficient level, and for this reason the oocytes do not resume meiosis. However, after fusion of such oocytes with meiotic oocytes (entering metaphase I) or mitotic blastomeres (arrested in mitosis), the nuclei of immature oocytes are capable of responding by rapid premature chromosome condensation.

3. The cytoplamic factor is not division-type specific. The factor from the meiotic cells (oocytes) causes chromosome condensation in the mitotic cells (interphase blastomeres) and vice versa.

4. The factor is not species specific — at least not between the mouse and bank vole species.

5. After parthenogenetic activation of mature oocytes, the cytoplasmic factor responsible for chromosome condensation quickly disappears, or is inactivated.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (599—600)

EFFECTS OF CELL CONTACT ON POSTTRANSFER DEVELOPMENT DURING CONJUGATION OF *TETRAHYMENA THERMOPHILA*

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Because ciliates consist of two types of nuclei: macronuclei and micronuclei, and because only macronuclei are phenotypically expressed, they can accumulate in their micronuclei many mutations and gross chromosomal abnormalities, which are not selected against during somatic life, and are not expressed until the sexual process — conjugation. These micronuclear defects which are expressed during conjugation can be useful for study of nuclear development, cell contact and fertility during conjugation. Some unfertile lines, with gross defects in their micronuclei have been known for a long time, are called "star" lines, and marked with "*". These lines are unable to produce functional generative pronuclei, however they are able to receive migratory pronucleus, when mated to another fertile line. After unilateral transfer, single pronuclei in both donor and recipient cells are diploidized, but they do not complete their development during first round of conjugation. These cells maintain their old macronuclei and are capable to another round of conjugation, which is completely normal, and during this consecutive conjugation new functional macronuclei develop. This phenomenon is called "Genomic Exclusion" (for a review see [1, 3, 6]).

In the present study the unfertile strongly hypoploid line A* mt V was crossed to the normal fertile line of another mating type. Then the dynamics of pairing in this cross was examined and compared with the control cross of two fertile lines. It was found that pairs from the cross A* x tester were separating after approximately 7,5 hrs since beginning of pairing, while in the control cross cells were pairing for 11,5 hrs, that is A* pairs were pairing for 4 hrs shorter than the control pairs. All the experiments were performed at a temp. of 30°C. Then the temporal pattern of development was examined in both the A* x tester and in the control crosses. For this cell samples containing pairs were fixed at

different times with Giemsa stain according to Bruns and Brussard [3], Martindale et al. [4].

It was found that in a cross A* x tester, the meiosis, 3rd pregamic division and unilateral transfer in a non-star mate, were proceeding at the same time as in a control one. In both cases, data on timing of stages were in good agreement with the data of Martindale et al. [4]. Then, however posttransfer development was completely blocked in both mates in 90% of cases. Only in about 10% of cases anlagens of new macronuclei were formed, but these pairs were not surviving because of any unknown developmental defect. However, some cases of apparently normal development of haploid nuclei which correspond to partenogenesis are known in *Tetrahymena* [5], and it is unlikely that failure of fertilization of a cross A* line with a normal tester, is a cause of the observed block in posttransfer development. Instead, it is very likely that premature separation of cells is a cause of this block. If this conclusion is correct, then it is expected that maintaining of the normal cell contact is critical for posttransfer development in *Tetrahymena*.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (601—602)

EFFECTS OF NOCODAZOLE ON MEIOSIS AND POSTMEIOTIC DEVELOPMENT IN A CILIATE *TETRAHYMENA THERMOPHILA*

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A *Tetrahymena thermophila* cell (a ciliate) is easy to handle and feasible in studying of meiosis because, it is easy to induce and to control in this species synchronous conjugation in mass samples. This is because conjugation triggers meiosis [6], and meiosis in *Tetrahymena* is a relatively rapid process compared to other meiocytes, including even yeast. It lasts about 4 hrs at 30°C [6].

Nocodazole (ND) is a potent antitubulin drug [2]. It affects mitosis [3, 4], and its action on meiosis in *Tetrahymena* is similar to that induced by colchicine in *Lilium* [5, 7].

In our study prestarved cells of complementary mating types were mixed at 30°C following the procedure of Martindale et al. [6] and pairs were formed in 1,5-2 hrs after that. Then ND was applied at different times after cell mixing. Treated and untreated (control) cell samples containing pairs were fixed at different times and stained with Giemsa stain according to Bruns and Brussard [1], Martindale et al. [6].

The main results of our study may be summarized as follows:

1. ND induced appearance of restitutive nuclei due to inhibition of chromosome movements during anaphase and telophase of both meiotic divisions. ND, however did not block separation of kinetochores. If ND was applied at stages corresponding to pachytene or later, then 5 bivalents were observed during metaphase I and next 10 diads, and finally 20 uni-chromatid chromosomes. These uni-chromatid chromosomes were despiralizing, and restitutive nuclei of interphase appearance were observed.

2. If ND was applied during early prophase of meiotic division at the prepachytene stages, then 10 monovalents instead of 5 bivalents were observed in metaphase I. Next, these chromosomes were again

fully splitting into 20 uni-chromatid chromosomes, and restitutive nuclei were formed as expected, irrespective of whether bivalents were formed or not. Apparently, Nocodazole inhibits pairing of chromosomes, most likely because of interfering with formation of synaptonemal complexes during prepachytene stages. So far it is the first case of experimental induction of achiasmatic nuclei in ciliates.

3. Restitutive nuclei received after ND treatment were undergoing an additional round of endoreplication, because following interphase approximately 40 uni-chromatid chromosomes were seen in many of them (exact counts were not available). This number corresponds to $8n$ ($n = 5$). The additional round of endoreplication in restitutive nuclei corresponds to the third pregamic division during normal conjugation. This 3rd division is a mitotic division of one of the haploid products of meiosis (for a review of normal stages of development see [6]). Thus restitutive nuclei progress in their development according to the general scheme of the developmental programme during conjugation in the presence of ND.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (603—606)

MODIFICATION IN FORMATION OF CILIARY STRUCTURES IN MIRROR-IMAGE DOUBLE CELLS OF THE CILIATED PROTOZOAN *PARAUROSTYLA WEISSEI*

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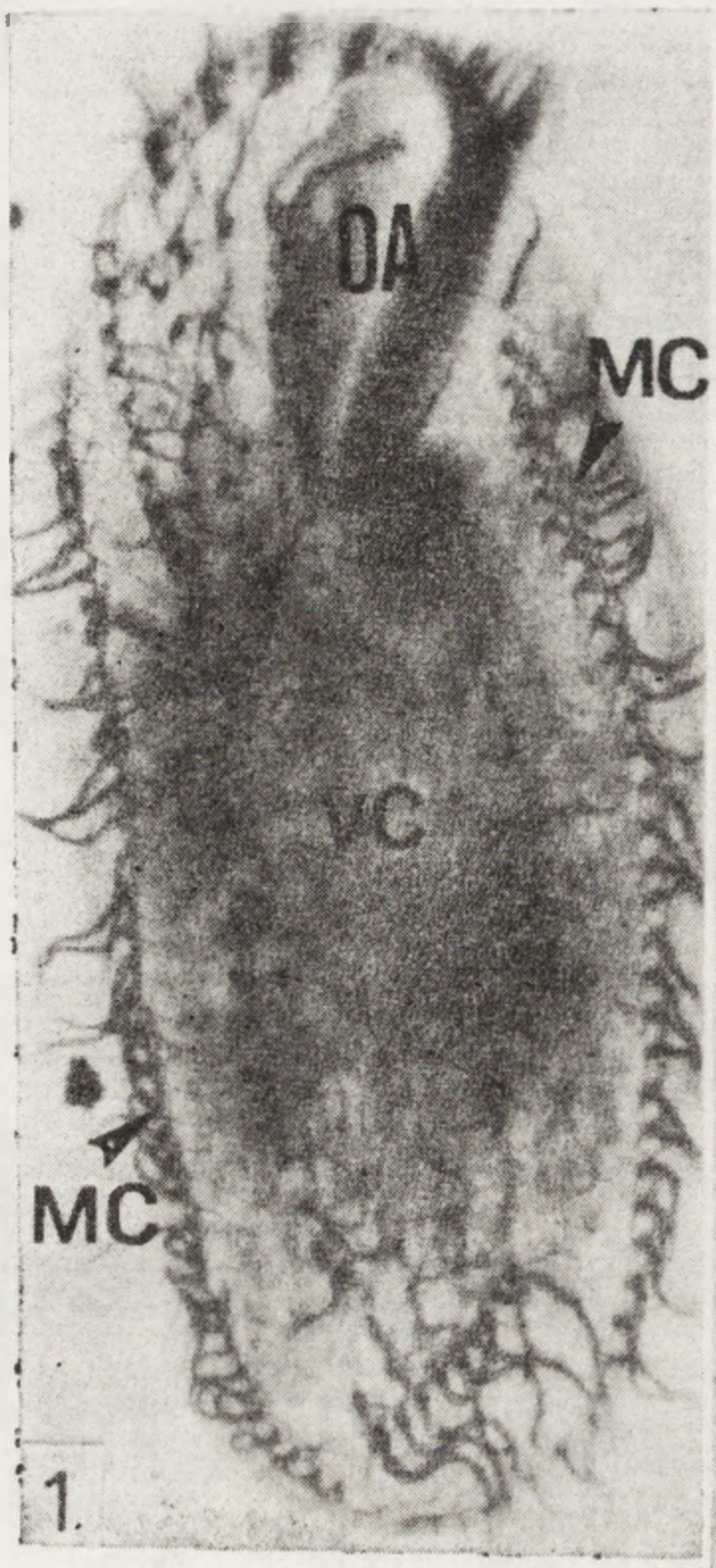
Ciliated protozoans are unique experimental systems due to the fact that cell lines with modified ciliary patterns can be produced either by genetic substitutions, or by artificial juxtapositions of specific cellular parts. The mirror-image symmetry homopolar twin cells of hypotrich ciliate *Paraurostyla weissei* are formed by two cellular entities fused side-by-side [3]. The left component (standard symmetry half — ST) shows normal complement of cortical structures comparable to that in single cells (Fig. 1), except the lack of the right marginal cirri at the fusion region (Fig. 2). In the right component (symmetry reversal half — SR) the overall ciliature is arranged in a mirror-image pattern of the normal half. The right margin of the SR half is covered by one, two or three rows if marginal cirri. In about 17% of a population, marginal cirri with inverted polarity occur in the SR half of the double. These abnormal configurations allow studying propagation of extra marginal rows in cells with affected overall pattern.

Light microscopic and transmission electron-microscopic studies of developing double cells revealed several deviations of normal morphogenesis of marginal cirri. They are as following:

1. De novo formation of marginal primordial streaks. This way of streak formation violates the standard morphogenesis in two ways: firstly, the initial streak formation does not involve prior disaggregation of old cirri present in proper cortical region, and secondly, the streak is formed at the left (not right — as in normal morphogenesis) side of the original marginal row.



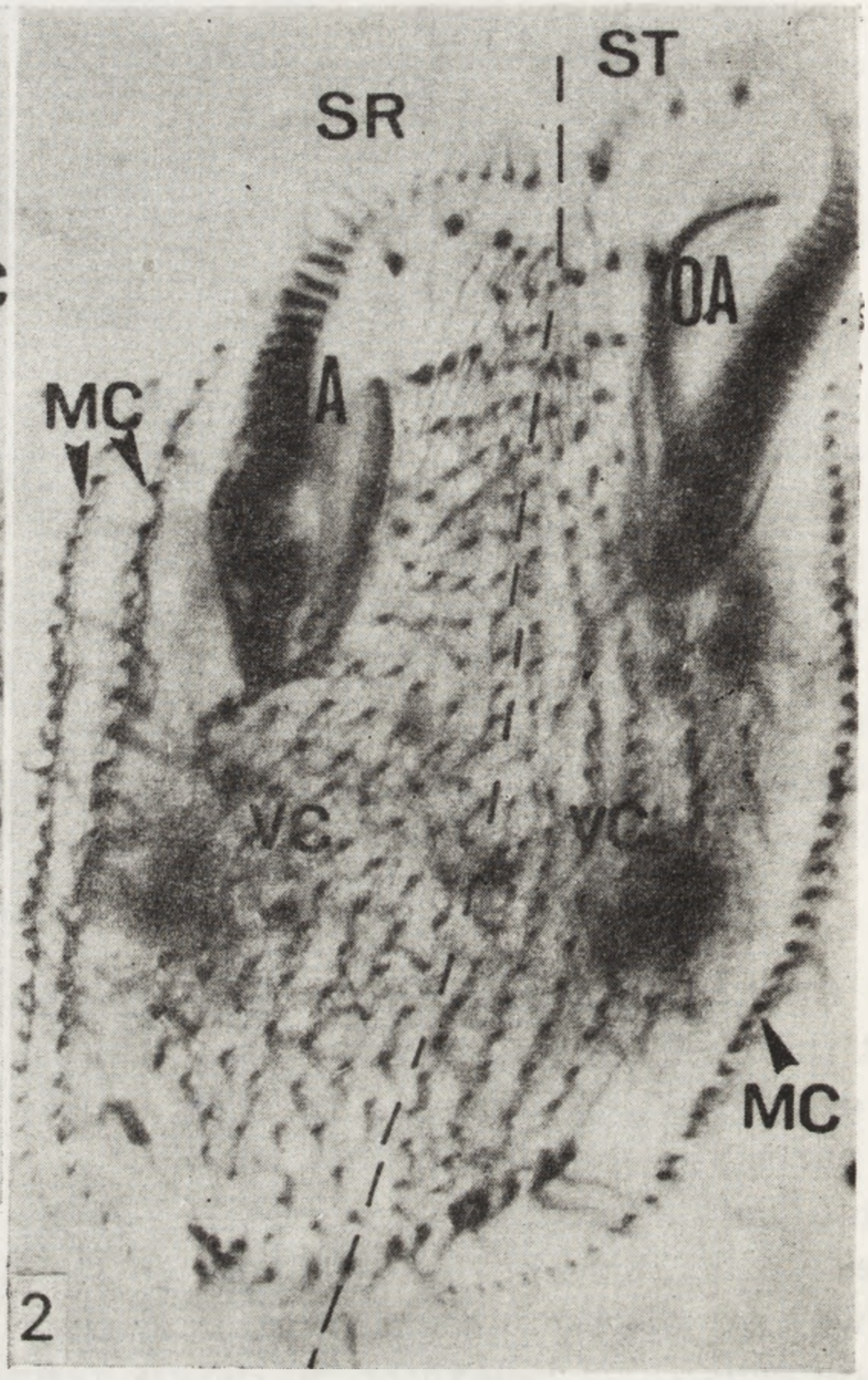


Fig. 1. Ventral side of single cell of *P. weissei* stained with Protargol
Fig. 2. Ventral side of the mirror-image doublet cell of *P. weissei* stained with Protargol. Vertical broken line represents the plane of symmetry

Abbreviations: OA — oral apparatus, MC — marginal cirri, VC — ventral cirri, SR — symmetry reversal half, ST — standard symmetry half

2. Formation of overlapping segments of marginal primordia. The morphogenesis of primordia initially starts with disaggregation of several (3–4) old marginal cirri. The basal bodies migrate from the cirri and from short "strings", but instead of joining end-to-end they overlap. Short strings migrate out from the parental row not only along its right side (as always occurs in normal morphogenesis), but also out to the left side. In effect, two primordial ciliary streaks can be formed on both sides of a parental marginal row.

3. Formation of inverted rows of marginal cirri. Inverted marginal cirri may develop according to the two aforementioned patterns, except

that the pairs of basal bodies constituting the ciliary streaks [2] are rotated 180°.

4. Morphogenetic inactivity of some of the old marginal rows. In many doublet cells in which there are 2 or 4 parallel rows of marginal cirri at the "proliferative zone" [1], usually only the internal row (closer to the oral apparatus) participates in morphogenesis, whereas old cirri from the second or third more external rows remain developmentally quiescent and are resorbed in later stages of morphogenesis.

The main conclusion derived from this study is that the cytotactic propagation of marginal cirri is seriously affected in the symmetry reversal half of the doublet of *P. weissei*. Modifications in the arrangement of marginal cirri in the symmetry reversal half results from modified pathways of morphogenesis of these structures, which in turn are regulated by overall patterning systems, partly dependent on cellular axes.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (607—610)

GENETIC BASIS OF ABERRATIONS IN THE CORTICAL PATTERN IN mlm MUTANT OF THE HYPOTRICH CILIATE *PARAUROSTYLA WEISSEI*

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A morphologically abnormal line of *Paraurostyla weissei* was isolated as a result of back-crosses between lines collected from natural sources. This "multi-left-marginal"(mlm) line possesses multipleid rows of marginal cirri on its left side. This is accompanied by changes in cell proportion [Figs. 1 and 2], increased frequency of cortical reorganizations and substantial decrease in the growth rate [1]. It was postulated that the observed modifications result from a single gene mutation. The purpose of this study was to verify this hypothesis.

Cells of *P. weissei* undergo a total conjugation [2] giving rise to a single zygocyst. During several days, the zygocysts develop into single cells which then divide forming clones. In order to make a cross, two lines of complementary mating types were mixed, and after firm pairs were formed, they were isolated singly into depression slides. Abnormal lines were distinguished by slower growth rate and aberrations in cell shape. They were then stained with Protargol, and if the multiplications in marginal cirri were found, they were scored as mlm lines.

The results of genetic analysis are presented in Table 1. Two independent crosses of two heterozygous lines isolated previously [1] were performed. In both cases, the obtained ratio of normal lines and mlm lines was 3 : 1. This suggested that the studied phenotype is dependent on a single gene. To confirm this result two more crosses were performed. A mutant line derived from a cross E4 × E5 was crossed to a non-parental, wild type normal line N1 collected freshly from a pond. The whole progeny was phenotypically normal but genotypically probably heterozygous. In a test cross one line derived from F1 was

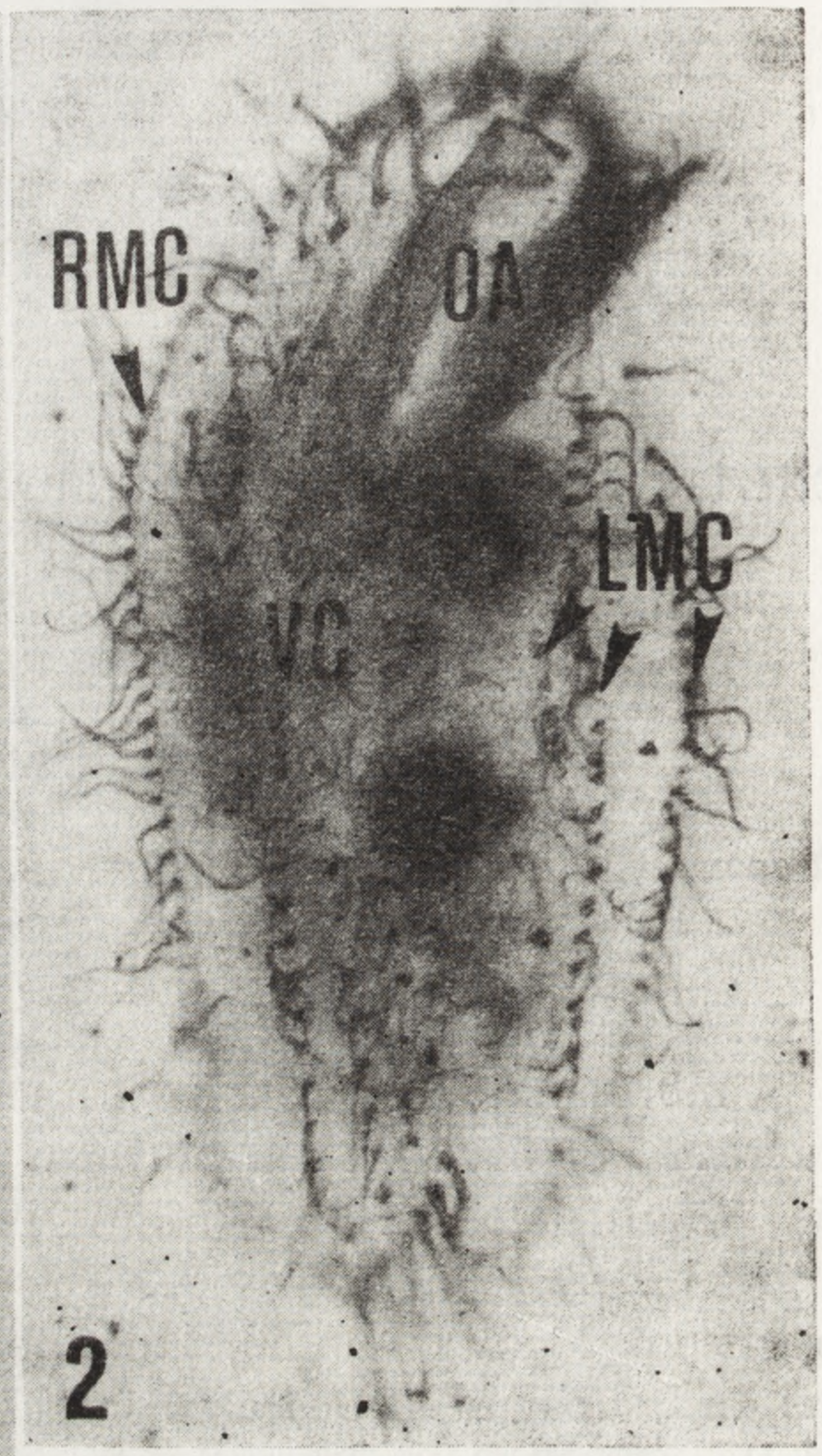

Fig. 1. The ventral surface of Protargol-stained cell of normal line of *Paraurostyla weissei* (× 550)

Fig. 2. The ventral surface of Protargol-stained cell of mlm line of *P. weissei* (× 550)

Abbreviations: OA — oral apparatus, VC — ventral cirri, RMC — right marginal cirri, LMC — left marginal cirri

crossed to parental line B16 (mlm/mlm × mlm/+). In the progeny the phenotypic ratio was 1 : 1.

The results of crosses obtained so far and the analysis of the progeny lines, allows us to conclude that the mlm phenotype is controlled by a recessive allele termed mlm at a single locus.

The mlm mutant of *Paraurostyla weissei* is a unique material for the study of inheritance of cortical pattern in hypotrich ciliates, and also for studies on the development of cortical structures in genetically modified morphological conditions.

TABLE 1

Genetic interpretation of control of mlm phenotype

Postulated genotype of original parents E4: mlm/+ E5: mlm/+ Interpretation of crosses				
Cross Genotype of parents	predicted results		observed results	p
	genotype	phenotype		
E4×E5 mlm/+ × mlm/+				
1	25% +/+ 50% mlm/+ 25% mlm/mlm	75% normal 25% mlm	84 normal 24 mlm*	$p>0.5$
2	25% +/+ 50% mlm/+ 25% mlm/mlm	75% normal 25% mlm	38 normal 13 mlm	$p>0.9$
B16×N1 mlm/mlm×+/+	100% mlm/+	100% normal	17 normal	—
B16×(B16×N1) mlm/mlm×mlm/+	50% mlm/+ 50% mlm/mlm	50% normal 50% mlm	12 normal 11 mlm	$p>0.8$

* 7 lines from this sample were not stained.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (611—612)

CALCIUM CONTENT AND LOCALIZATION IN MEROISTIC INSECT FOLLICLE

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In studies on the activation of the egg cell, the role of calcium in this process is one of the main problem. A transient rise in ionic calcium released from the intracellular store, appears to be the first detectable signal of metabolic derepression after fertilization or parthenogentic activation [3, 5]. The phenomenon has been observed in egg cells of animals from various systematic groups, however, not in the insects. We approached the problem by studying accumulation of calcium in the developing oocyte of *Galleria mellonella*, a representant of *Lepidoptera* [2]. By using X-ray microprobe analysis, it has been found that the concentration of calcium in the oocyte is 3-fold higher than in the trophocytes and in the follicle cells in postvitellogenic follicle. Concentration of calcium in previtellogenic follicle approximately equals that in the postvitellogenic ones, amounting to 9 and 10 mmoles per 1000g of wet weight tissue respectively.

The two-dimensional and "on line" registration of X-ray generated calcium signals, suggested that distribution of calcium coincides with accumulation of the yolk globules within the ooplasm. This suggestion has been supported by results gained with the two different precipitation methods: either with sodium oxalate [1], or with N,N-naphtalylhydroxylamine [4]. In oocytes treated by these reagents, abundant electron opaque deposits, indicating the presence of calcium, were observed on the periphery of the lipid yolk globules. The reaction products were also seen in the ooplasm as the delicate vesicular structures which frequently attached to the lipid yolk globules. It seems likely that the structures represent some kind of intracellular calcium transporting system which supplies the calcium to the oocyte yolk.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (613—616)

PENETRATION OF 4′, 6-DIAMIDINE-2-PHENYLINDOLE [DAPI] A DNA LIGAND, INTO THE NUCLEAR APPARATUS OF LIVING CELLS OF *PARAMECIUM OCTAURELIA*

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DAPI, related to berenil, was synthesized by Dann [2] as a potential drug against trypanocide. This drug was found to be one of the most sensitive fluorophores, commonly used for detection, as well as for quantitative analysis of DNA, both spectrophotometrically in solutions and by fluorescence microscopy of cell structures [1, 3]. The drug concentration necessary to obtain a bright fluorescence in structures containing DNA proved to be very low. Thus, cells suspended in such low DAPI concentrations did not reveal any readily perceptible changes in their behaviour or metabolism, thereby making it possible to follow the passage of the drug in vitally active cells [4]. *Paramecium aurelia*, a free-living protozoan cell with a well-formed digestive system, presented a good model for tracing the uptake of the drug from the medium and its subsequent penetration into the nuclear apparatus.

The cells of *Paramecium octaurelia*, strain 299, grown in axenic medium [5], were collected from four-day-old cultures and carefully washed with 5.0 mM tris-HCl buffer with 1.0 mM $CaCl_2$, pH 7.6, hereafter called T-buffer.

A Zeiss Ergaval fluorescence microscope with a HBO 50 lamp was used for fluorescence observations.

Striking differences were found in the rates of penetration of DAPI into the nucleus of the living cell, depending upon the composition of the surrounding medium. The nuclei of cells incubated in T-buffer-DAPI mixture showed incipient fluorescence within five minutes. Fluorescence developed during the following 10 minutes. On the other hand, the nuclei of cells incubated in axenic medium began to fluorescence only after three to four hours. Comparison of DAPI penetration rates

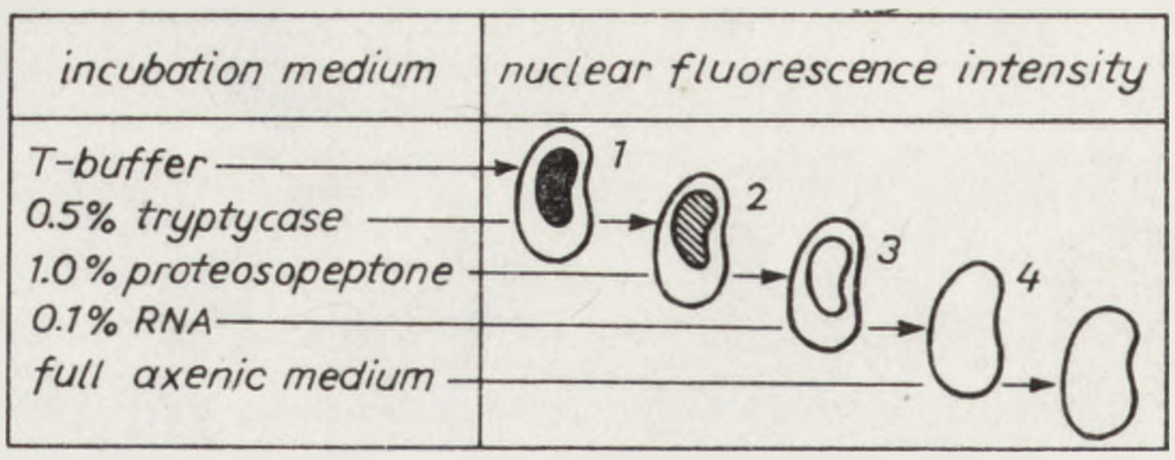

Fig. 1. DAPI penetration into living cells of *Paramecium octaurelia* depending on the composition of the incubation medium. Washed cells were suspended in full axenic medium or in reaction mixture containing particular components of medium dissloved in T-buffer. DAPI concentration 2 μg/ml, incubation time 15 min.; *1* — intensive, *2* — medium, *3* — weak, *4* — no fluorescence in nuclei

into the nuclei of cells incubated in individual components of axenic medium dissolved in T-buffer, showed that RNA was mainly responsible for retardation of drug penetration (Fig. 1). Drug penetration accelerated when starved cells were used instead of well-fed cells, as well as when the cells were incubated at 30°C instead of at 10°C (Fig. 2).

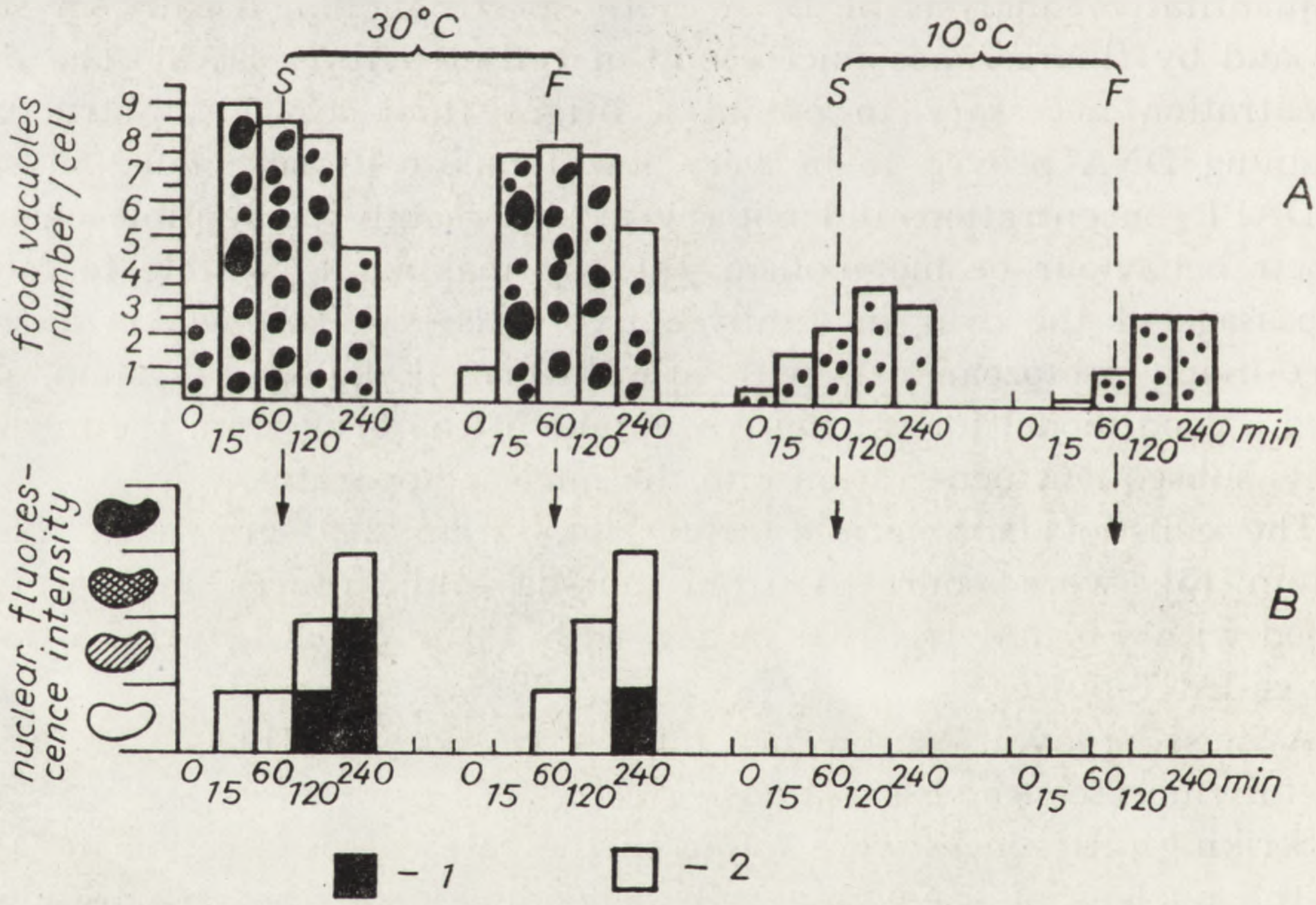

Fig. 2. Correlation between (*A*) food vacuole formation, and (*B*) penetration of DAPI into the nucleus as a function of time. F — well-fed cells or S — cells starved for 18 hours, were incubated in 0.1% buffered RNA with Dow Latex particles (original suspension diluted 200 times), and 1.6 μg of DAPI/ml. Incubation temperature 30°C or 10°C. Fluorescence can be detected: *1* — in all cells, *2* — only in part of cells

When the latex particles were suspended in the incubation mixture, a bright green fluorescence of food vacuoles, replete with latex particles, was observed as early as five minutes after incubation began. The nucleus was invisible at that time. After two to four hours of incubation, food vacuoles — still full of latex particles — changed fluorescence colour to light gray and hardly differ from the background, while the nucleus fluoresced bright green. In some cases, especially when cells were incubated in crude commercial RNA from BDH (contaminated with about 1% of DNA) or in 0.001% DNA, no nuclear fluorescence could be detected, even after 24 hours of incubation in DAPI. However, numerous bright green fluorescing globules containing latex particles were egested as undigestible contents of food vacuoles.

It can be concluded from these studies that DAPI and probably other similar positively charged low-molecular-weight compounds, when diluted in water or salt solution, penetrate into the protozoan cells via food vacuoles without any limitation by an unmediated diffusion mode. However, it seems that polymers contained in a culture medium, linking the drugs by ionic bonds, are the main barrier protecting cells against drug penetration. Negatively charged polyanions such as DNA, RNA and some proteins or polypeptides, serve as such a barrier against DAPI penetration. If the drug complexed with the polymers is to pass to the nucleus, it is first digested in a food vacuole. If it is not digested, it will be egested subseqently outside the cell.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (617—620)

REGENERATION OF CILIA IN *TETRAHYMENA* PRETREATED WITH DNA-LIGAND 4,6-DIAMIDINE-2-PHENYLOINDOLE (DAPI). EFFLUX OF THE LIGAND

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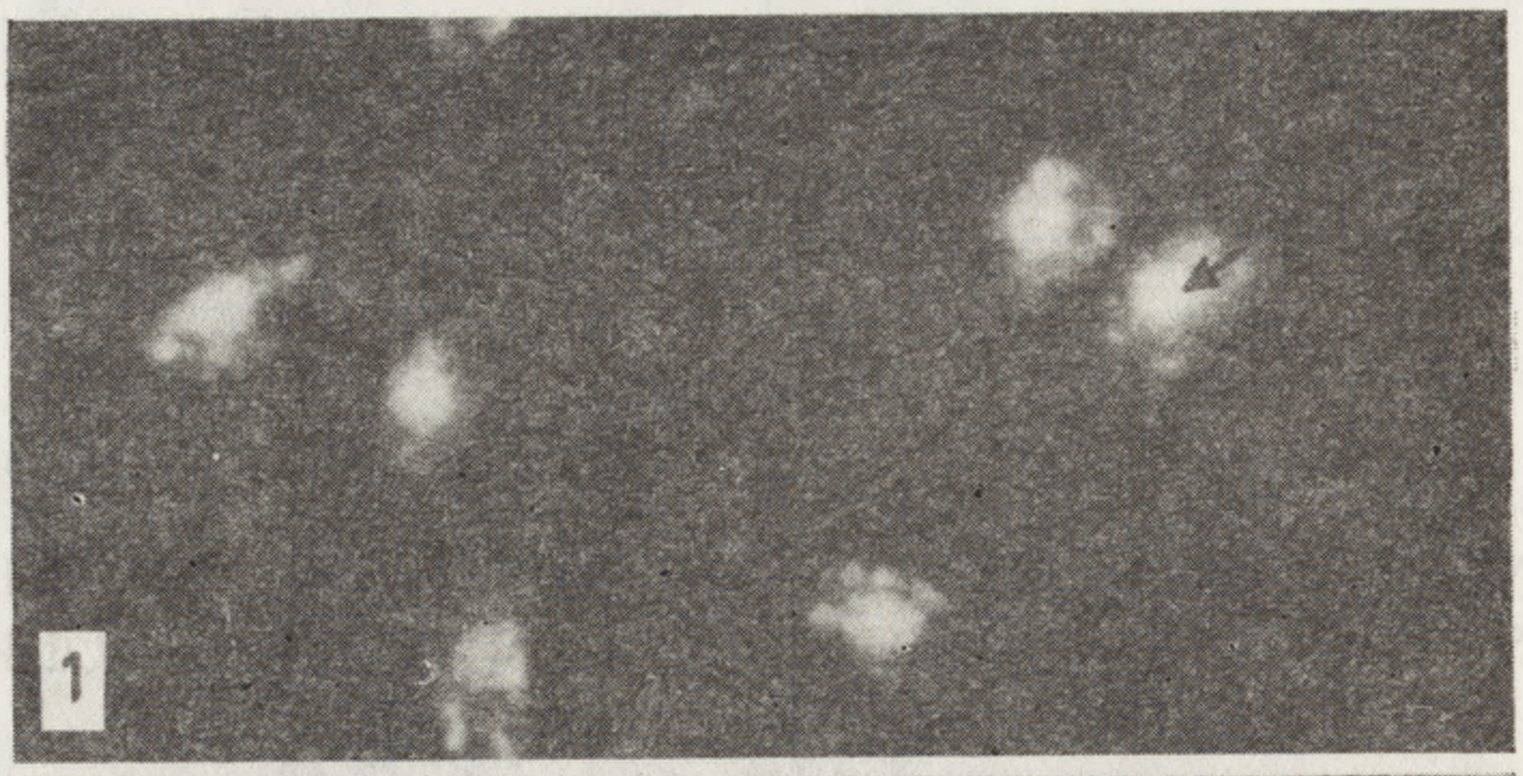
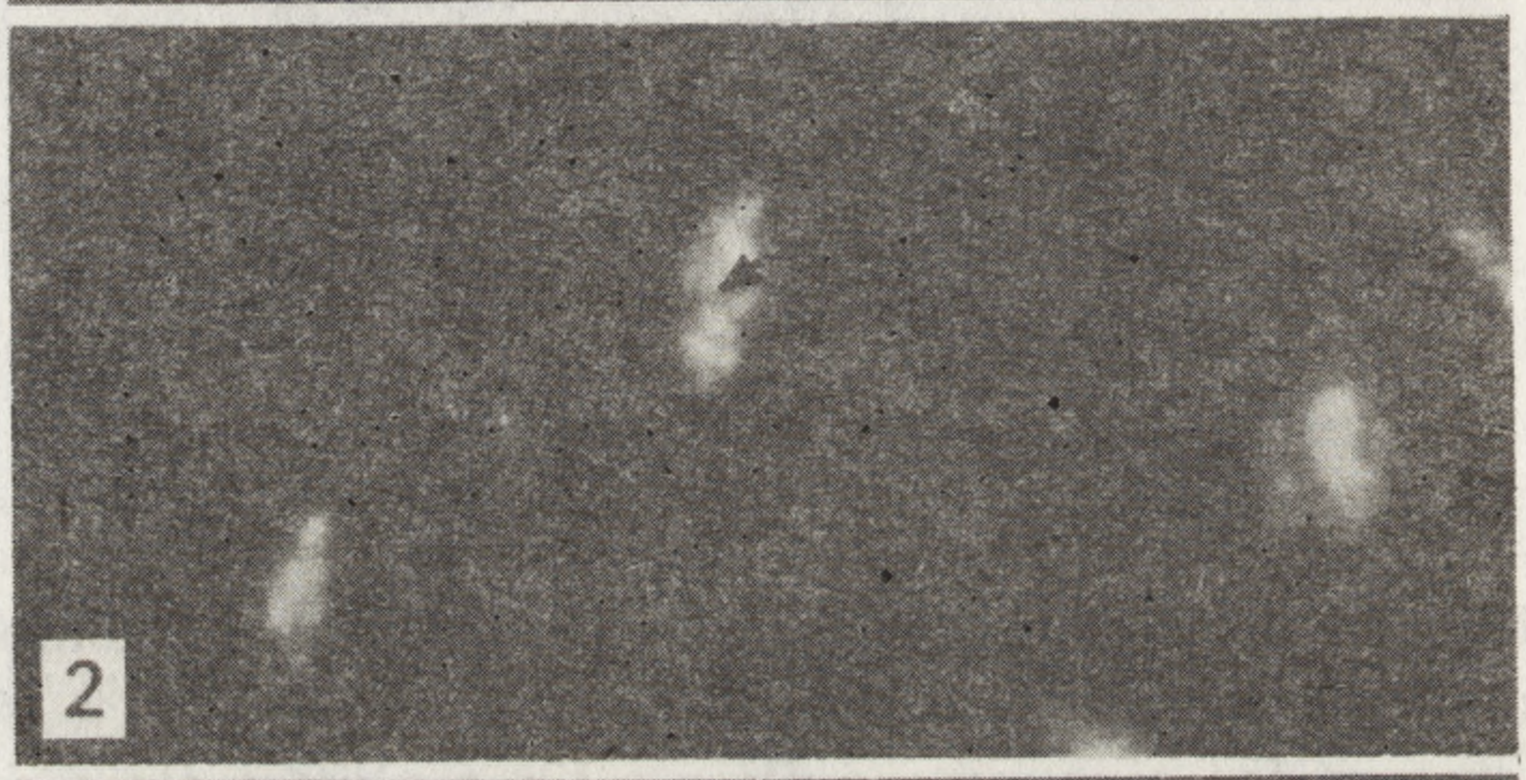
It is known that deciliation of *Tetrahymena* cells stimulates synthesis of RNA and proteins. These newly synthesized compounds are utilized for supplementation of the intracellular pool which is the main source of protein for cilia regeneration. When this pool is insufficient, for example, after long-time cell starvation, the newly synthesized proteins are also used for cilia reconstitution [1, 2, 5].

It was reported that deciliated cells of *Tetrahymena* (therefore motionless) restored their mobility during about 40 min after cilia amputation [1, 2, 5]. However, when the cells were treated for 20 h with a DNA-ligand DAPI, before deciliation, their cilia regeneration lasted 30 min longer as compared with the control [4]. Moreover, in the cells starved in the presence of DAPI, there was no stimulation of RNA synthesis after deciliation [4]. Therefore, the lag period of cell reciliation was supposed to be a result of DAPI-template inhibition. Transferring the cells into DAPI-free medium seems to be necessary for releasing template from the ligand inhibition, and subsequent activation of RNA and protein synthesis.

The presence of the ligand in the cells can be easily observable under the fluorescent microscope, due to the formation of fluorescent DAPI-DNA complexes [3].

Figure 1 presents fluorescence of *Tetrahymena* cells incubated during 20 h in starvation buffer (50 mM Tris-HCl, pH 7.2) supplemented with DAPI in concentration of 1 μg/ml, just before cilia amputation. The intensive fluorescence of macronuclei (Ma) as well as undefined cytoplasmic structures can be seen. After 70 min of cilia regeneration





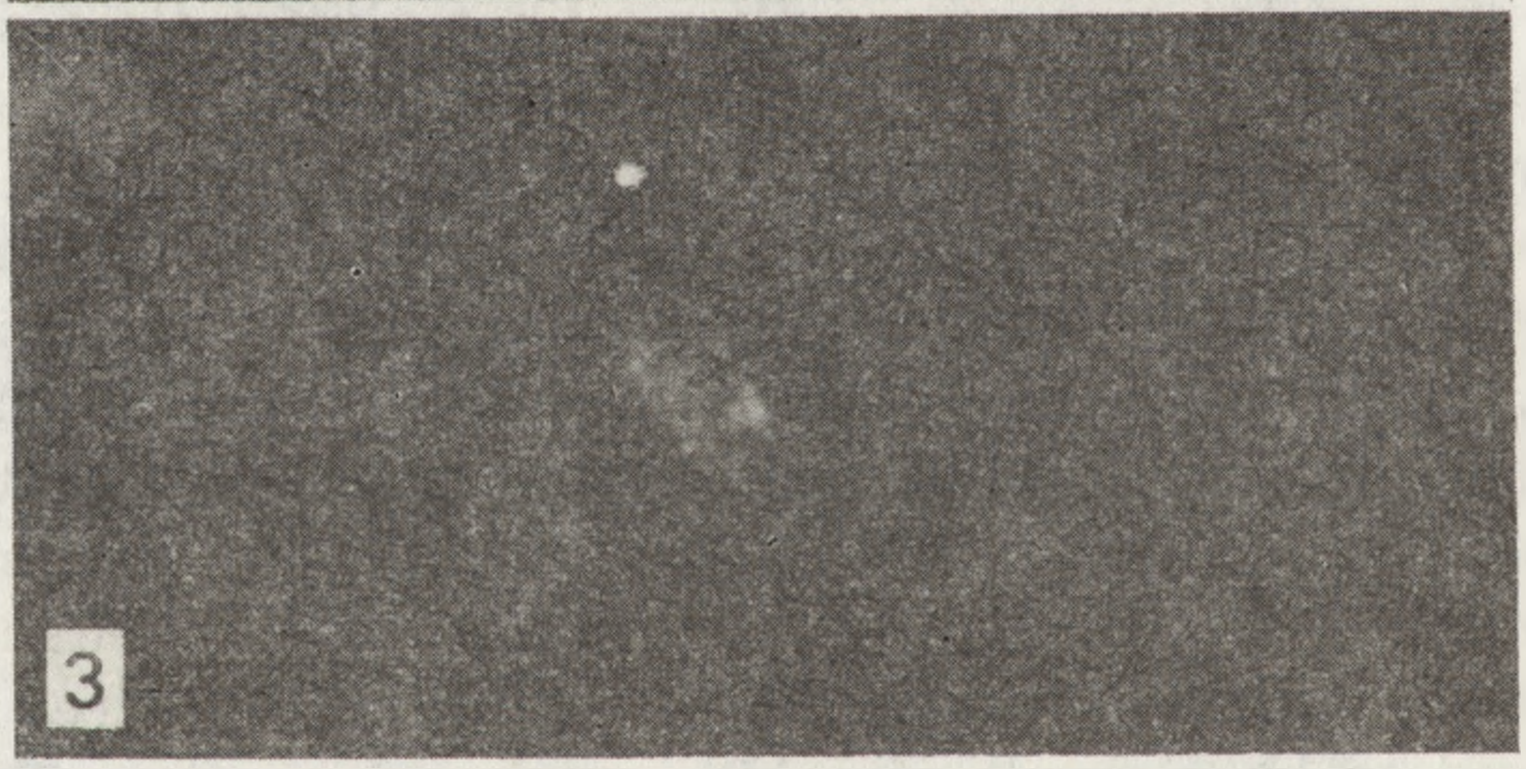


Fig. 1-3. Fluorescence of *Tetrahymena* cells treated with DAPI
Fig. 1. After 20 h of incubation just before cilia amputation
Fig. 2. After 70 min of cilia regeneration
Fig. 3. After 120 min of cilia regeneration

Abbreviation: arrow — macronucleus. The decrease of fluorescence intensity of the macronuclei can be noted

in DAPI-free medium, the fluorescence intensity is much lower (Fig. 2) than at the moment of deciliation (Fig. 1). After 120 min of reciliation, the fluorescence of macronuclei continues to decrease (Fig. 3). It should be mentioned, however, that even several hours after the completion of the process of cilia regeneration, the fluorescence of the macronuclei remains noticeable.

The decrease of fluorescence intensity of cells suggests partial efflux of DAPI from the cells. The efflux seems to be maximal during the first hour after transferring the deciliated cells into DAPI-free buffer.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (621—624)

EFFECTS OF SELECTED β-LACTAME ANTIBIOTICS ON *TETRAHYMENA PYRIFORMIS* GL. I. INFLUENCE ON CULTURE GROWTH AND ACTIVITY IN CULTURE CONDITIONS

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Earlier investigations showed that penicillin, in sublethal concertrations, inhibited cell multiplication of *Tetrahymena pyriformis* GL [1, 2, 3]. The results supported the presumption that penicillin interferes with cellular metabolism as the nonspecifical inhibitor. Penicilin G, penicillin V, ampicillin and cloxacillin (POLFA) were the selected compounds showing different chemical modifications of the β-lactame chemical group. It is interesting whether differences in chemical composition may be responsible for different biological effects exerted on eucaryotic cells.

The effects of the above selected penicillins on the dynamics of the culture growth of *Tetrahymena pyriformis* GL were investigated. Experimental cultures were grown axenically in "PPY+S" medium, as described elsewhere (Rebandel — see besides). Samples were taken periodically during each experimental procedure, and cells were counted electronically. Lag phases after addition of each antibiotic (final concentr. 10 mM) to exponentially growing cultures were compared. Experimental cultures (shallow and aerated) were inoculated with cells from exponentially growing stock, 3 hrs. before treatment with antibiotics. Controls were supplemented with 1 ml of distilled water (the volume of antibiotic solution added) which appeared to be ineffective.

Addition of similar molar concentrations of tested compounds evoked different effects (Tab. 1). The longest lag phase was produced by cloxacillin, and next by ampicillin, while penicillin G was ineffective in used concentration. The lag produced by cloxacillin was as long as the time needed for doubling of cell number in control cultures (2.5 h). The effects of two different concentrations (2 mM and 10 mM) of each antibiotic were compared in cultures growing under optimal and suboptimal conditions for gaseous exchange (culture depths: 0.53 cm and

TABLE 1

Lag phases produced by tested antibiotics (10 mM) in exponentially growing cultures

Antibiotic	Duration of the lag period, in hours
Penicillin G	0
Penicillin V	0.5
Ampicillin	1.5
Cloxacillin	2.5

1.11 cm respectively) as described elsewhere (Rebandel — see beside). Different rates of cell multiplication during the phase of exponential growth, were obtained as the effect of both kinds and concentrations of antibiotic (Tab. 2). The strongest were the effects of cloxacillin and ampicillin. Decrease of maximal densities reached in treated cultures were related to concentration, but not to he kind of compound (Tab. 3). Only for penicillin G, the effect of concentration was higher in deep than in shallow cultures. It could be possible that not all of the physiological processes related to cell cycle and to culture growth efficiency, are relatively irrespective to differences in chemical composition between tested compounds.

Estimation of specific antibacterial activity of given compounds, seems to be the best way for determination of their real concentration (as native substance) in solution. The bacteria *Staphylococcus aureus* strain ATCC 25923 (specific sensitivity against penicillin = 0.1 i.u.), was used as the biological indicator. Cultures of *Tetrahymena pyriformis* GL growing with different concentrations of tested antibiotics, were sampled periodically. Samples of cell-free medium (obtained by filtration (MILIPORE) after centrifugation) were serially diluted, and dilutions were inoculated quantitatively with bacteria suspension. After 18 hrs. of culti-

TABLE 2

Coefficients of cell number growth during 1 hour in exponentially growing cultures after treatment

Experimental conditions	A*		B*	
	I**	II**	I**	II**
Penicillin G	0.82	0.84	0.71	0.59
Penicillin V	0.87	0.83	0.75	0.77
Ampicillin	0.89	0.78	0.68	0.50
Cloxacillin	0.79	0.56	0.69	0.47
control	1.12		0.78	

TABLE 3

Maximal densities reached in treated and control cultures (in values $\times 10^3$ cells per ml)

Experimental conditions	A*		B*	
	I**	II**	I**	II**
Penicillin G	760	668	633	465
Penicillin V	815	742	607	561
Ampicillin	870	591	542	361
Cloxacillin	731	682	529	478
control	866		640	

* A, B — 0.53 and 1.11 cm (respectively) — depths of the cultures.

** I, II — 2 and 10 mM (respectively) — concentrations of antibiotics in experimental cultures.

vation, the threshold dilution of each sample of *Tetrahymena* exhausted medium, capable of limiting bacteria growth, was estimated.

No changes of antibacterial activity in samples of the medium taken periodically from protozoan cultures were observed irrespectively of kind of tested antibiotic. All compounds showed stability in culture conditions. The titers of samples were stabile for up to 96 hrs. of *Tetrahymena* growth. It seems that none of the antibiotic could be taken up or metabolised by protozoan cells, and that the end-products of *Tetrahymena* metabolism (liberated by cells into the culture medium) don't show any β-lactamaze-type activity.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (625—628)

EFFECTS OF SELECTED β-LACTAMIC ANTIBIOTICS ON *TETRAHYMENA PYRIFORMIS* GL. II. INFLUENCE OF PREINCUBATION ON FOOD VACUOLE FORMATION DURING DIRECT AND RECOVERY EXPERIMENTS

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It was pointed out by Nilsson [1] and by Rasmussen [2], that presence of particulate material (even inedible) in the medium appears to be sufficient to induce formation of food vacuoles in *Tetrahymena*. On the other hand, the intensity of the process of phagocytosis is linked to cellular metabolism and can be regulated chemically. Thus, the induction and intensity of this process are a relatively independent phenomena, and can be investigated separately. In earlier investigations, penicillin lowered intensity of food vacuole formation in *Tetrahymena* [3, 4]. It was suggested that penicillin could interfere with some processes of cellular metabolism important for proper efficiency of phagocytosis, however some adaptation-type responses were also presumpted. It seems to be interesting, whether the differences in chemical composition of selected antibiotics in the penicillin group (penicillin G, penicillin V, ampicillin and cloxacillin) would be responsible against the intensity of the particulate food uptake by the cell. Effects of previous or prolonged treatment on the immediate action of each of the selected compounds, were compared.

Experiments were carried out on *Tetrahymena pyriformis* GL, growing in shallow cultures in "PPY + S" medium (Rebandel — see besides) in optimal temperature (28°C). Food vacuole formation was induced mechanically with India ink suspension (ROTRING) during 10 minutes, and then cells were fixed with neutralized 1% formaldehyde. The mean number of food vacuoles formed by 100 cells in each sample was the measure of intensity of the process of phagocytosis. Two types of experiment were made. In "prolonged action" experiments, cells were cultivated with antibiotic during 18 hrs. (first dose), and then a second dose was added 5 minutes prior to the test of phagocytosis. Samples which were untreated

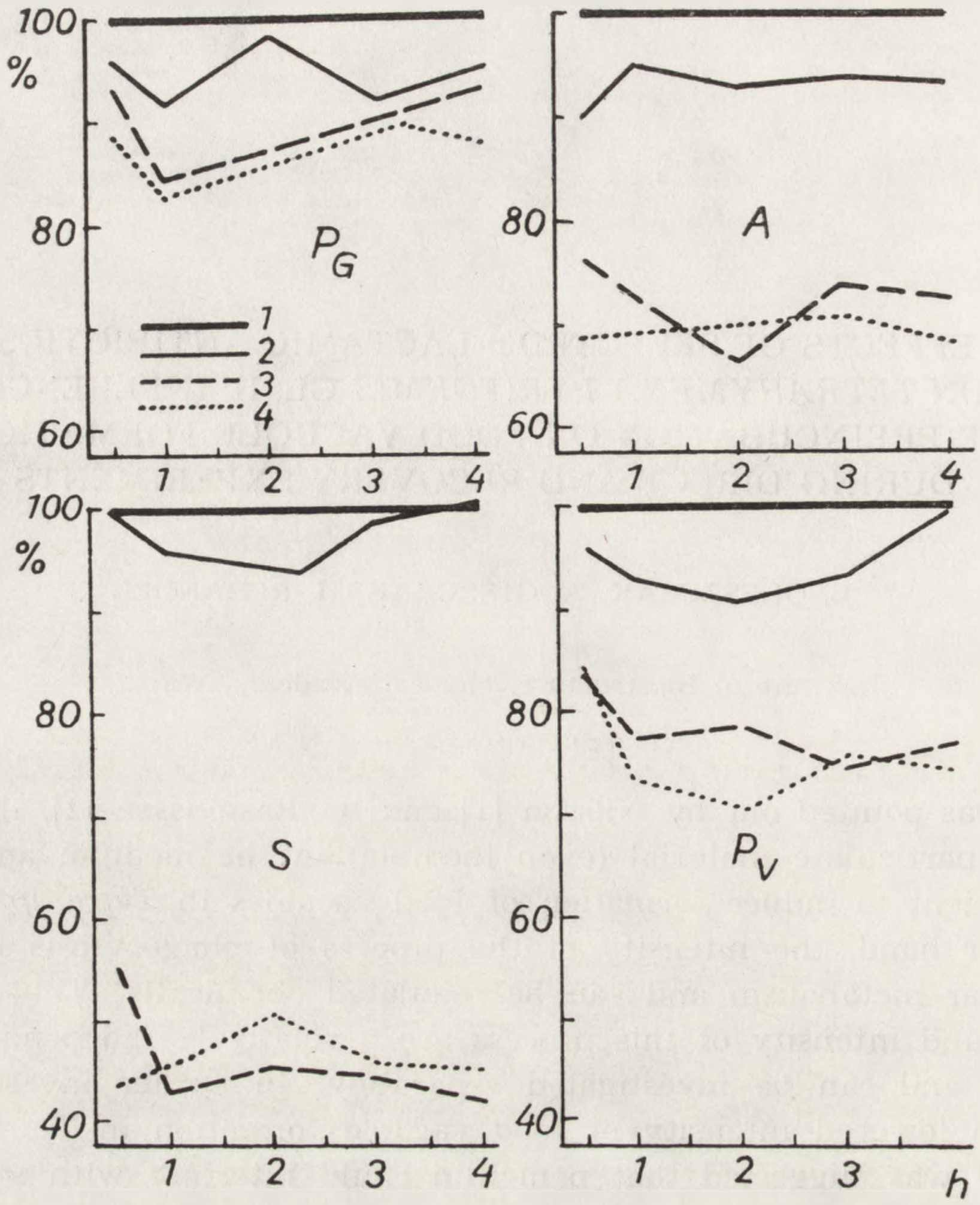

Fig. 1. Changes of intensity of food vacuole formation in *Tetrahymena* treated with antibiotics after transfer of the cells into fresh culture medium. Responses of previously treated (during 18 hrs. of cultivation) and naive cells are compared in different time points after shifting down of cells into fresh medium. Abscissa: time in hours. Ordinate: percentage decrease of intensity of food vacuole formation — 100% — untreated controls. P_G — penicillin G, P_V — penicillin V, A — ampicillin, S — cloxacillin. P_G, P_V, A — 10 mM, S — 2.5 mM. Explanation of curves: *1*, *3* — cells non treated before washing, *2*, *4* — cells cultivated with antibiotics before washing, *3*, *4* — cells treated with antibiotics in different time points after washing, *1*, *2* — untreated cells after washing

and treated once, were the controls. In "previous action" experiments, cells were cultivated during 18 hrs. with antibiotic, and then transferred (centrifugation) into fresh culture medium (without antibiotic). In different time points after washing, the same antibiotic was added 5 minutes prior to the test of phagocytosis.

TABLE 1

Effects of antibiotics on intensity of food vacuole formation in naive and pretreated cells. P_G – penicillin G, P_V – penicillin V, A – ampicillin, S – cloxacillin

Concentration of antibiotics in mM			Mean numbers of food vacuoles			
during 18 hrs. of cultivation	given 5 min. before the test	total	P_G	P_V	A	S
–	–	–	7.72	7.77	7.84	7.82
10	–	10	7.56	6.38	7.61	5.14
–	10	10	8.04	7.43	7.89	5.30
10	10	20	7.65	7.32	7.03	4.59
–	20	20	6.25	6.82	6.97	0
10	20	30	6.61	7.12	5.92	0

The obtained results are shown (Tab. 1 and Fig. 1) separately for each type of experiment. In "prolonged action" experiments, pretreated cells were generally less sensitive than "naive" cells (previously untreated) against antibiotic added immediately before induction of phagocytosis (Tab. 1). The results indicate that dose response and effect of mode of application are different for tested compounds, however these differences seem to be less visible for penicillin G and penicillin V. In "previous action" experiments, cultivation of cells with each of the antibiotics seems to have no effect on their response to new doses of the same compound applied after washing (Fig. 1). It should be pointed out that in this kind of experiment, cell multiplication was reactivated less than 0.5 hrs. after washing. Pretreated cells responded similarly as untreated (naive one) against immediate treatment with the compound, however only pretreated cells showed slightly decreased phagocytosis, in spite of absence of antibiotic during induced food vacuole formation. In all the experiments, cloxacillin was strongest, and ampicillin was stronger than penicillin G and penicillin V. Generally, the obtained results visualise different strength, and slight but essential differences in mode of action between the compared antibiotics.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (629—630)

ADAPTATION OF *TETRAHYMENA PYRIFORMIS* GL TO COLISTIN ON THE BASIS OF SELECTED PHYSIOLOGICAL FUNCTION

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The experiments involving the effects of inhibitors of cell metabolism on *Ciliata*, indicate that *Tetrahymena* adapts to the presence of low, non-lethal concentrations of inhibitors. Tetrahymena's reaction to the presence of poison in medium is defined as "physiological adaptation". Experiments have been carried out with colistin (sulphate). It is an antibiotic that lies in the cell membrane, and does not go beyond the surface to the cytoplasm. The effects of non-lethal concentration of colistin (0.05 mM/l and 1 mM/l) were investigated on the basis: multiplication rate, processes of cytokinesis, endocytosis and stomatogenesis.

The results were:

1. *Tetrahymena pyriformis* GL can adapt to used concentrations of colistin (physiological adaptation).

2. The disturbance of the physiological processes of the cells and the time of their renormalization is dependent on the concentration of colistin.

3. The time of the additional lag phase is proportional to the concentration of colistin.

4. The regulation of the cell cycle in the presence of colistin, is associated with the "set-back" in the cell cycle.

5. The process of resorption of oral primordium is characteristic for *Tetrahymena* ("all-or-none").

6. The concentration of colistin on synchronization of cell division in *Tetrahymena* has no effect.

POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (631—634)

THE EFFECT OF INDOMETHACIN ON CELL PROLIFERATION AND CONTENTS OF ESTROGEN AND PROGESTIN RECEPTORS IN UTERUS OF ESTRADIOL-TREATED IMMATURE RATS

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Immature castrated female rats were subcutaneously injected with estradiol — E_2 (3.3 μg in 0.2 ml of carrier, three times every 24 h), or indomethacin — I (2 × 1.5 mg in 0.2 ml of carrier per 24 h) or both (E_2 + I) in the same doses. Control rats received 0.2 ml of carrier (glycerol in saline containing 1 mg $NaHCO_3$ per 0.2 ml).

After 0; 24; 48; 72 h from the first injection, animals (6–8 rats) of the respective groups were sacrificed and the uterus was removed. In the uterus, the rate of ^{3}H-T incorporation into DNA and the contents of cytoplasmic receptors of estrogens (ER_c) and progestin (PR_c) were determined, in vitro.

For the determination of ^{3}H-T incorporation into DNA uterus specimens were incubated for 1 h in Eagle medium (at 37°C) containing ^{3}H-thymidine (1 μCi/ml) — spec. act. 5 Ci/mmol, in an atmosphere of 95% O_2 and 5% CO_2. Subsequently, the specimens were homogenized and washed with 5% perchloric acid, whereupon the precipitate was hydrolized with perchloric acid (15 min. 90°C), and in the hydrolyzates the radioactivity of tritium and deoxyribose content, were determined [1]. From the data, the amount of ^{3}H-T incorporated into uterine DNA was calculated. The contents of both receptors were assayed using the dextran-charcoal procedure [2]. ER_c was determined using ^{3}H-estradiol (10 nM) and DES (1000 nM), and PR_c — with the use of ^{3}H-R 5020 (10 nM) and R 5020 (1000 nM). Estradiol injected into rat females at 24 h intervals induced during the first 2 days a clear-cut increase in the rate of ^{3}H-T incorporation into uterine DNA (DNA synthesis) whereupon (after 72 h) this incorporation was markedly attenuated, though it still exceeded the control level (Fig. 1).

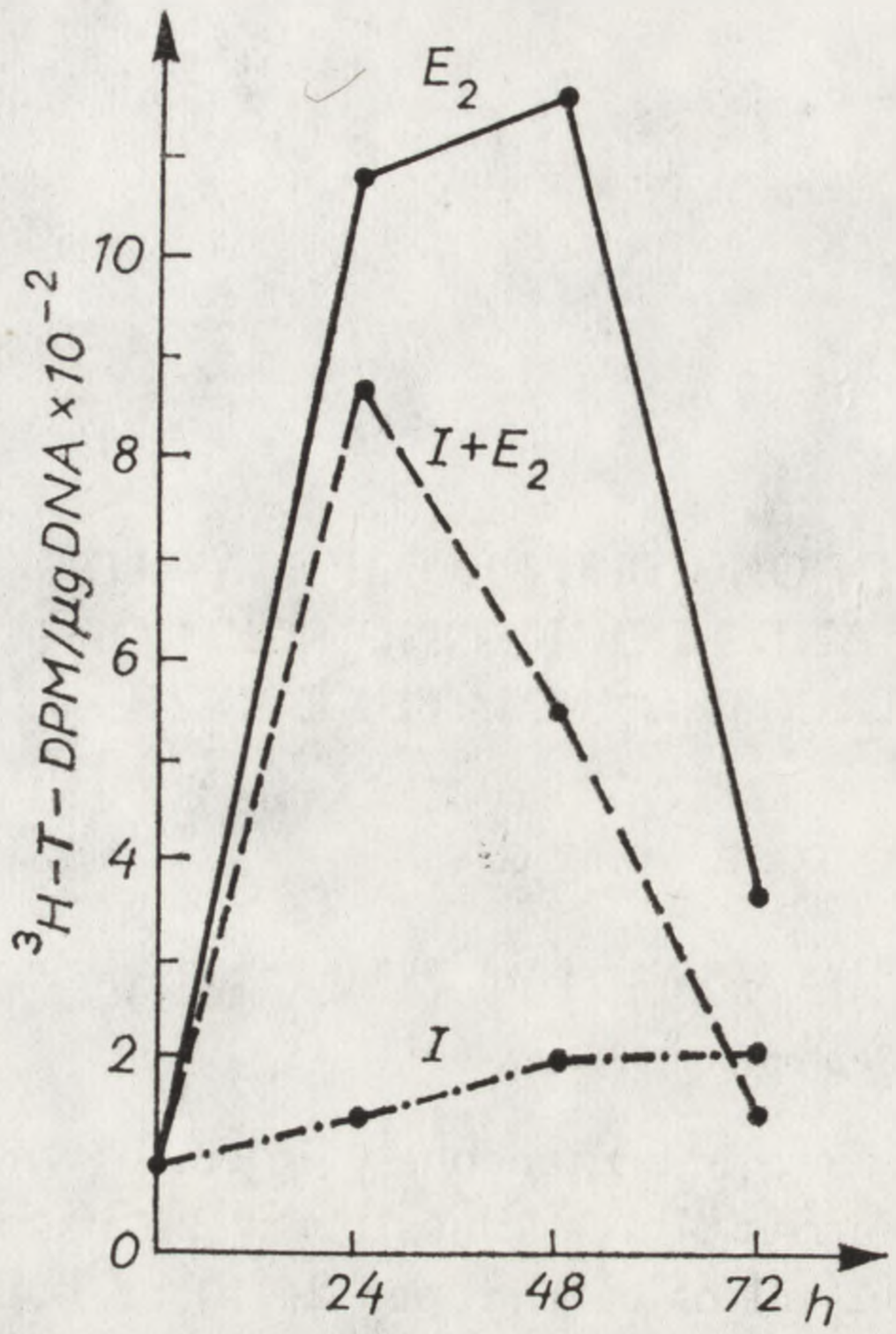

Fig. 1. The rate of ^{3}H-thymidine (^{3}H-T) incorporation into uterine DNA of rats treated with estradiol (E_2) indomethacin (I) or I + E_2

As compared with E_2, indomethacin did not by itself exert any evident effect on DNA synthesis in the uterus. When administered jointly with estradiol (E_2 + I), indomethacin substantially reduced the rate of ^{3}H-T incorporation into DNA. This effect was most expressed

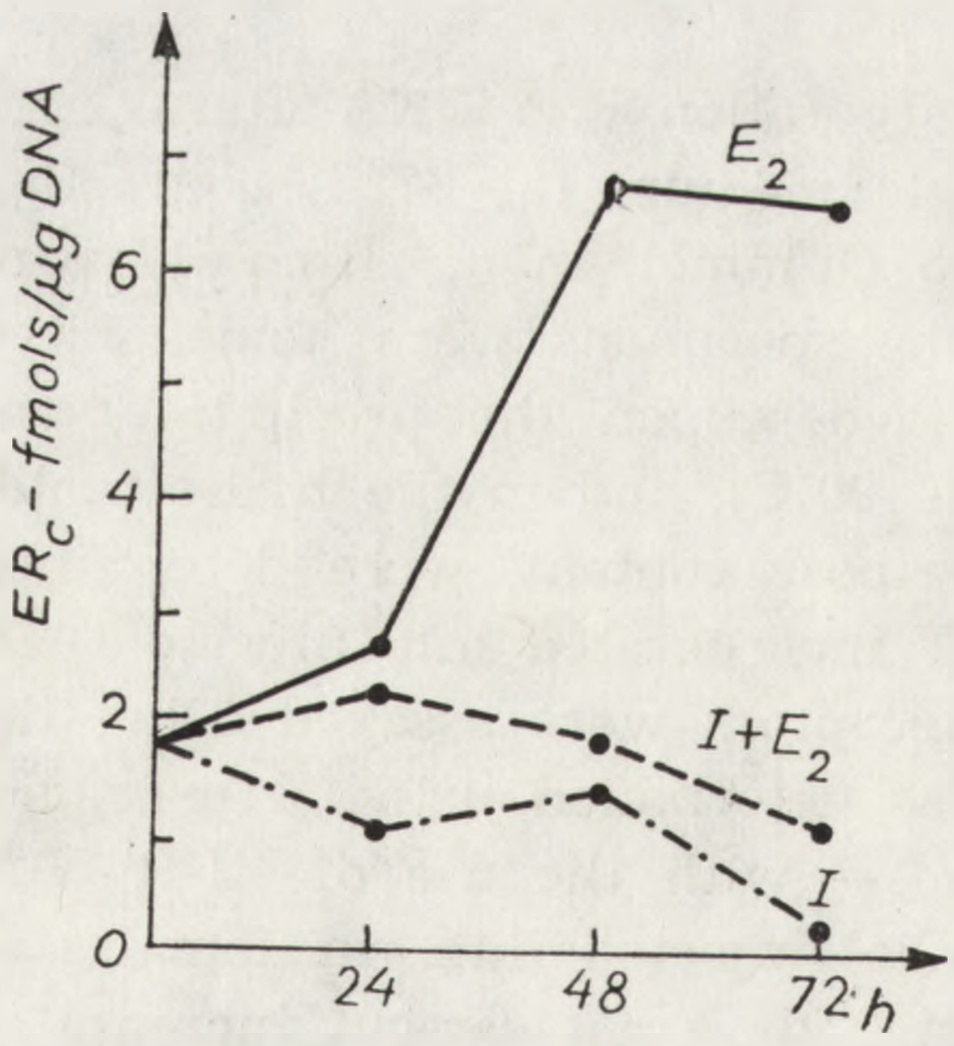

Fig. 2. The contents of estrogen receptor (ER_c) in rats uterus after injections E_2, I + E_2 or I

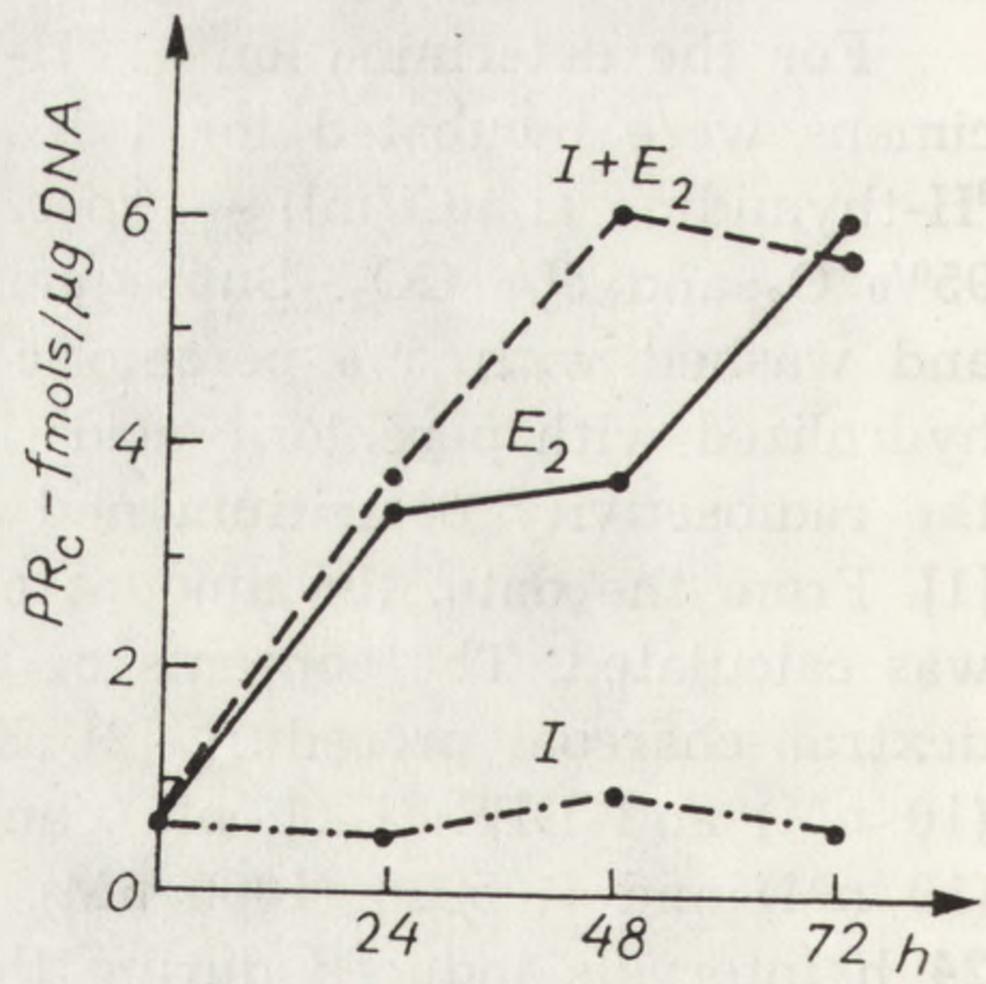

Fig. 3. The contents of progestin receptor (PR_c) in rats uterus after injections E_2, I + E_2 or I

in the 48-th and 72-nd h after the first injection of estradiol (Fig. 1). Estradiol injections induced an increase in the uterine ER_c and PR_c contents (Figs. 2 and 3). Indomethacin administered by itself during 3 days did not markedly influence the PR_c level, whereas it evidently lowered the ER_c concentration in the uterus in the 72-nd h of the experiment. Indomethacin applied jointly with estradiol did not exert any effect the estradiol-induced increase in the uterine ER_c and PR_c contents during the first 24-h period. The subsequent indomethacin injections (at 48 and 72 h) substantially inhibited the further estradiol-induced increase in uterine ER_c content, whereas they had no effect on the further increase in PR_c. The present results indicate that indomethacin administered for an appropriately long period can inhibit the estradiol-induced cell DNA synthesis and the increase in ER_c content of rat uterus, whereas it exerts no effect on the estradiol-induced increase in PR_c level.

It seems that the changes in the level of the cytoplasmic receptor of estrogens are correlated with uterine cell proliferation, while the synthesis of the cytoplasmic progestin receptor is regulated independently of cell divisions.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (635—638)

INVOLVEMENT OF GANGLIOSIDES IN DIFFERENTIATION OF N2a NEUROBLASTOMA CELLS

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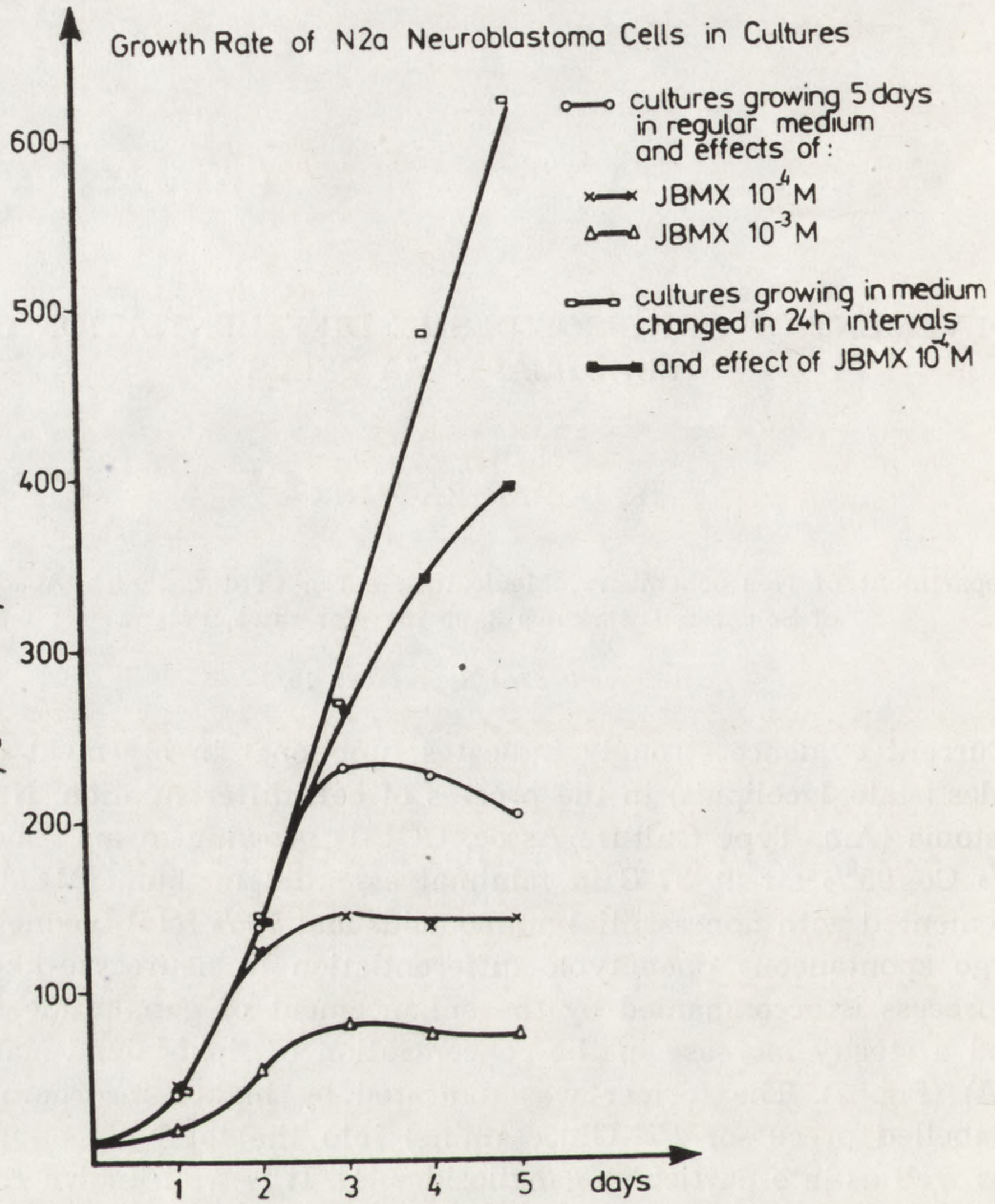
Current evidence strongly indicates functional involvement of gangliosides (sialoglycolipids) in the process of cell differentiation. N2a neuroblastoma (Am. Type Culture Assoc. CC131) growing in an atmosphere of 5% Co_2/95% air at 37°C in minimal essential medium (MEM-Eagle) supplemented with nonessential amino acids and 10% fetal bovine serum, undergo spontaneous phenotypic differentiation to neurocyte-like cells. This process is accompanied by the enhancement of ganglioside synthesis and a steady increase in the concentration of lipid-bound sialic acid (LBSA) (Fig. 2). The former was indicated by higher incorporation of radiolabelled precursor (^{3}H-Glucosamine) into the total glycolipid fraction as well as into particular gangliosides [1]. It is well known that N2a neuroblastoma in culture, shows a high degree of morphological and biochemical plasticity. For example, the process of differentiation can be stopped by changing the culture-growth medium every 24 hours. Under such conditions, the cultures grow significantly faster (Fig. 1), and the cells pile up and preserve the neuroblast like appearance through out whole observation period (5–7 days). In these fast-growing cells we did not observ any changes in the content and synthesis of gangliosides (Fig. 2), which is relevant to the concept of the straight involvement of these molecules in the cell differentiation process. Enrichment of the medium with exogenous gangliosides (Fig. 3) caused the opposite effect: a significant and dose-dependent inhibition in the growth of neuroblastoma cells, with a most advanced morphological differentiation. Recently, data showing that the gangliosides have receptor-as-



sociated binding capacities for many neurotropic substances, was collected. We can hypothetise, that the cells growing in culture may release the factor (s) promoting their further differentiation and stimulate the synthesis of membrane gangliosides as specific receptor molecules. Substances causing the elevation of c-AMP level (IBMX — isobuthylmethylxantine, cholera toxin, dibutyryl — cAMP) promote differentiation, inhibit growth and stimulate ganglioside synthesis of N2a neuroblastoma cells. Thus, it may be suggest, that the c-AMP system is involved in processing the signal responsible for cell differentiation and enhanced ganglioside synthesis.

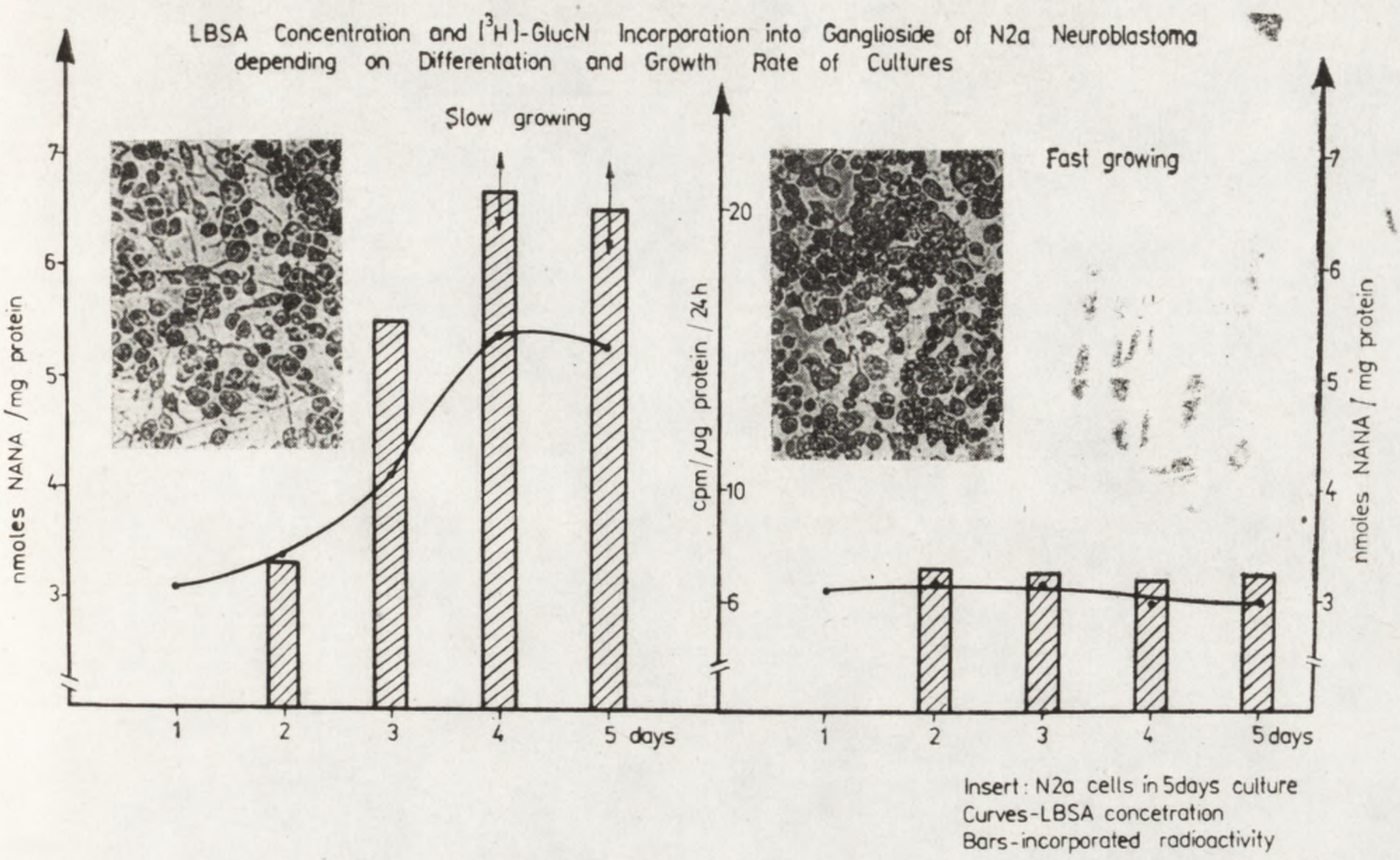


Fig. 2

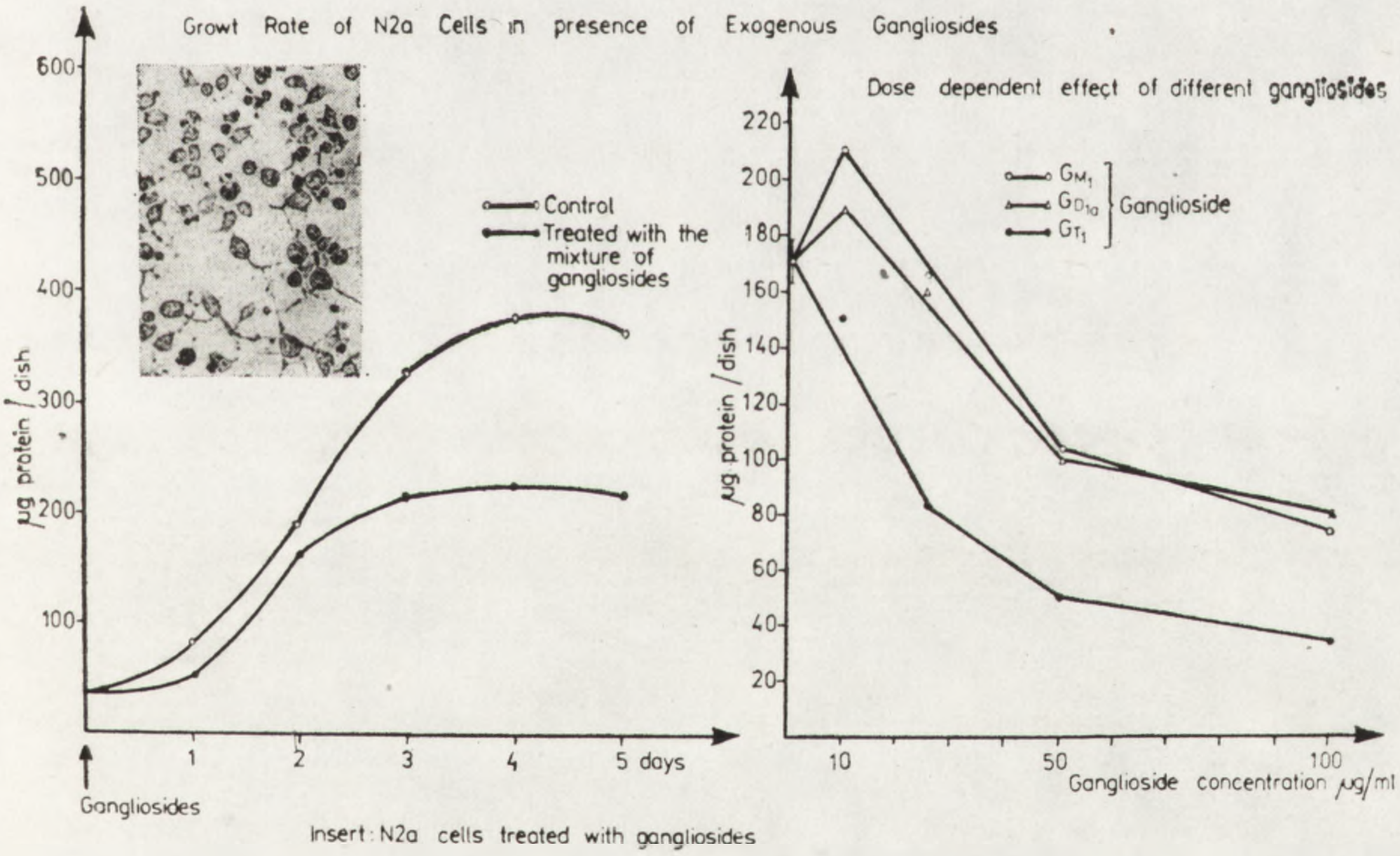


Fig. 3

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (639—644)

ENHANCEMENT OF PROTEIN AND LIPID GLYCOSYLATION IN 10 $T_{1/2}$ MURINE FIBROBLASTS BY RETINYL ACETATE

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Carcinogen induced neoplastic transformation in the C3H-10 $T_{1/2}$ CL8 (10 $T_{1/2}$) cell line is strongly inhibited by retinoids when these compounds are added prior to the phenotypic expression of transformation. The alterations of cell biological parameters suggested retinoid effects on membrane structure and function [1]. Addition of retinyl acetate (0.05 or 0.5 μg/ml) to normal non-transformed 10 $T_{1/2}$ fibroblasts inhibits the growth rate and saturation density of the cells (Fig. 1). Analysis of glycoproteins and glycolipids [2] revealed that as 10 $T_{1/2}$ cells progressed from logarithmic to confluent growth phase, incorporation of

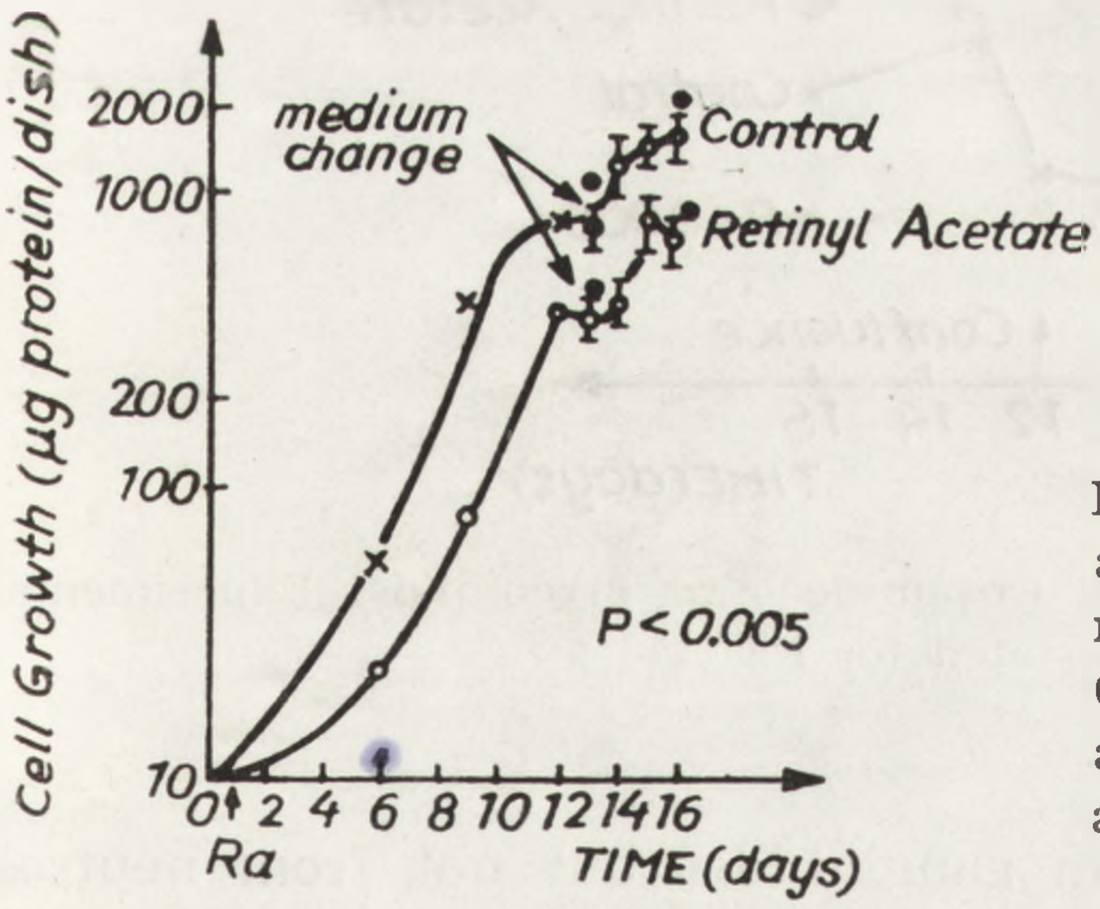

Fig. 1. Growth rate and reduced saturation density of 10 $T_{1/2}$ fibroblasts by retinyl acetate. Cultures were seeded in 60 mM dished in BME + 10% serum and after 24 hours received the retinyl acetate 0.1 μg/ml and then grown to confluence

^{3}H-Glucosamine into glycocompounds increased in the presence of 0.1 μg/ml retinyl acetate (Figs. 2 and 3). In addition to this increased synthesis, the time course studies on ^{3}H-Glucosamine labelled cells showed

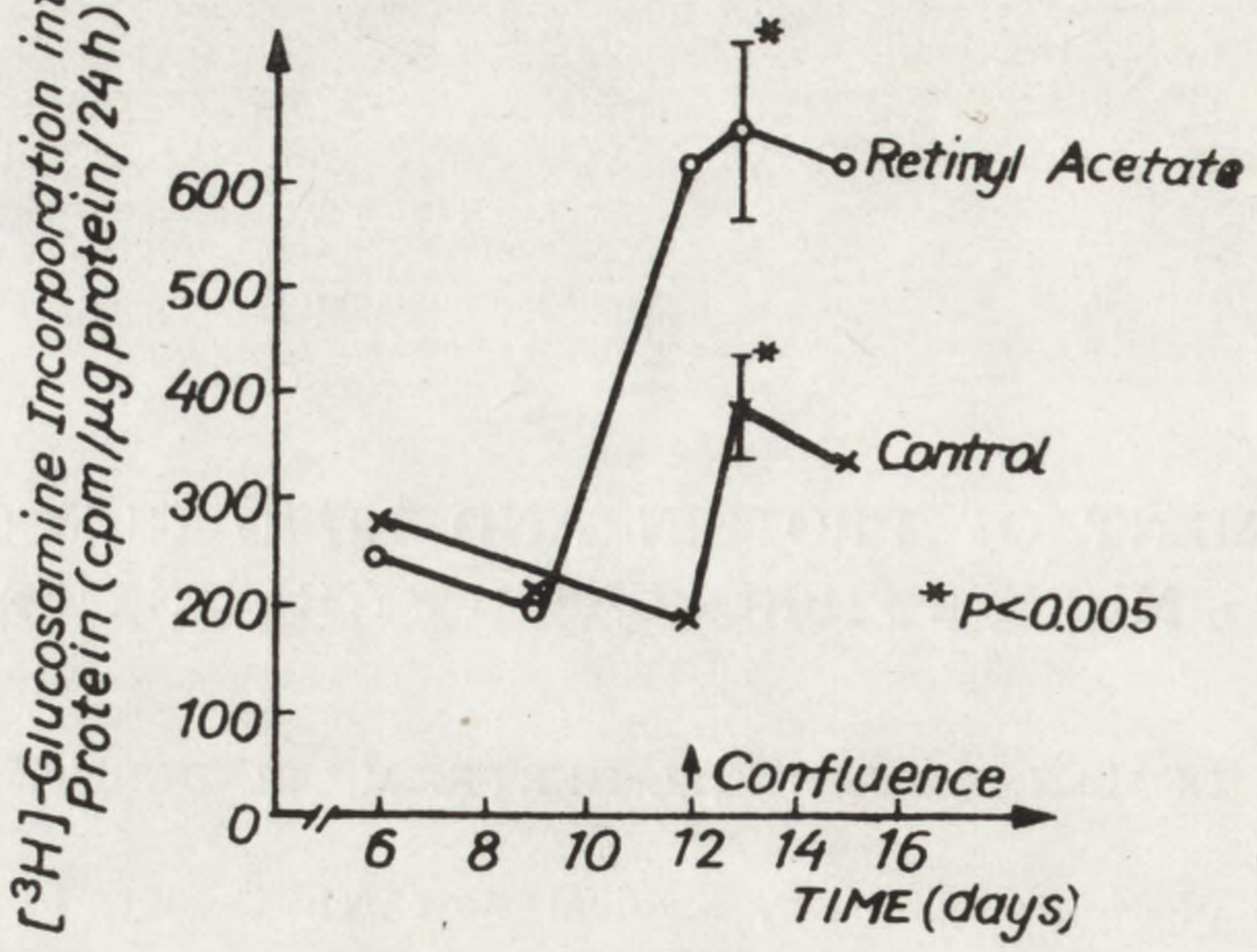

Fig. 2. Enhancement of ^{3}H-glucosamine incorporation into proteins of 10 $T_{1/2}$ fibroblasts growing in presence of 0.1 µg/ml retinyl acetate. The label 10 µCi/dish was applied into cultures 24 hours before harvesting

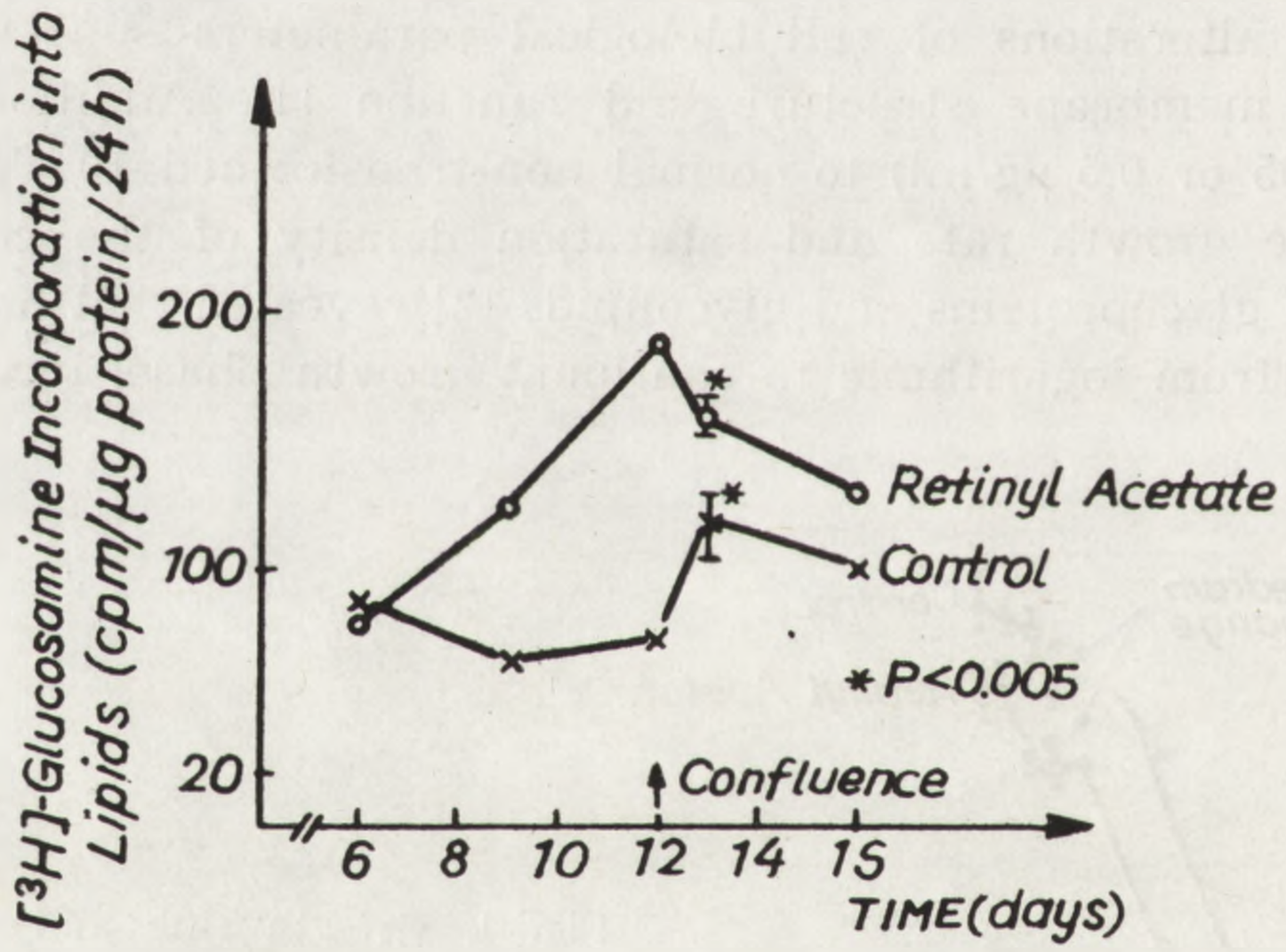

Fig. 3. Increased incorporation of ^{3}H-glucosamine into glycolipids. Experimental conditions as stated for Fig. 2

a slower rate of loss of label from gangliosides but not from neutral glycolipids and glycoproteins (Figs. 4 and 5). Analysis of gangliosides revealed that as 10 $T_{1/2}$ cells progressed from logarithmic to confluent growth phase, total gangliosides and the more complex gangliosides G_{D1b} and G_{D1a} increased in concentration. Treatment with 0.1 µg/ml

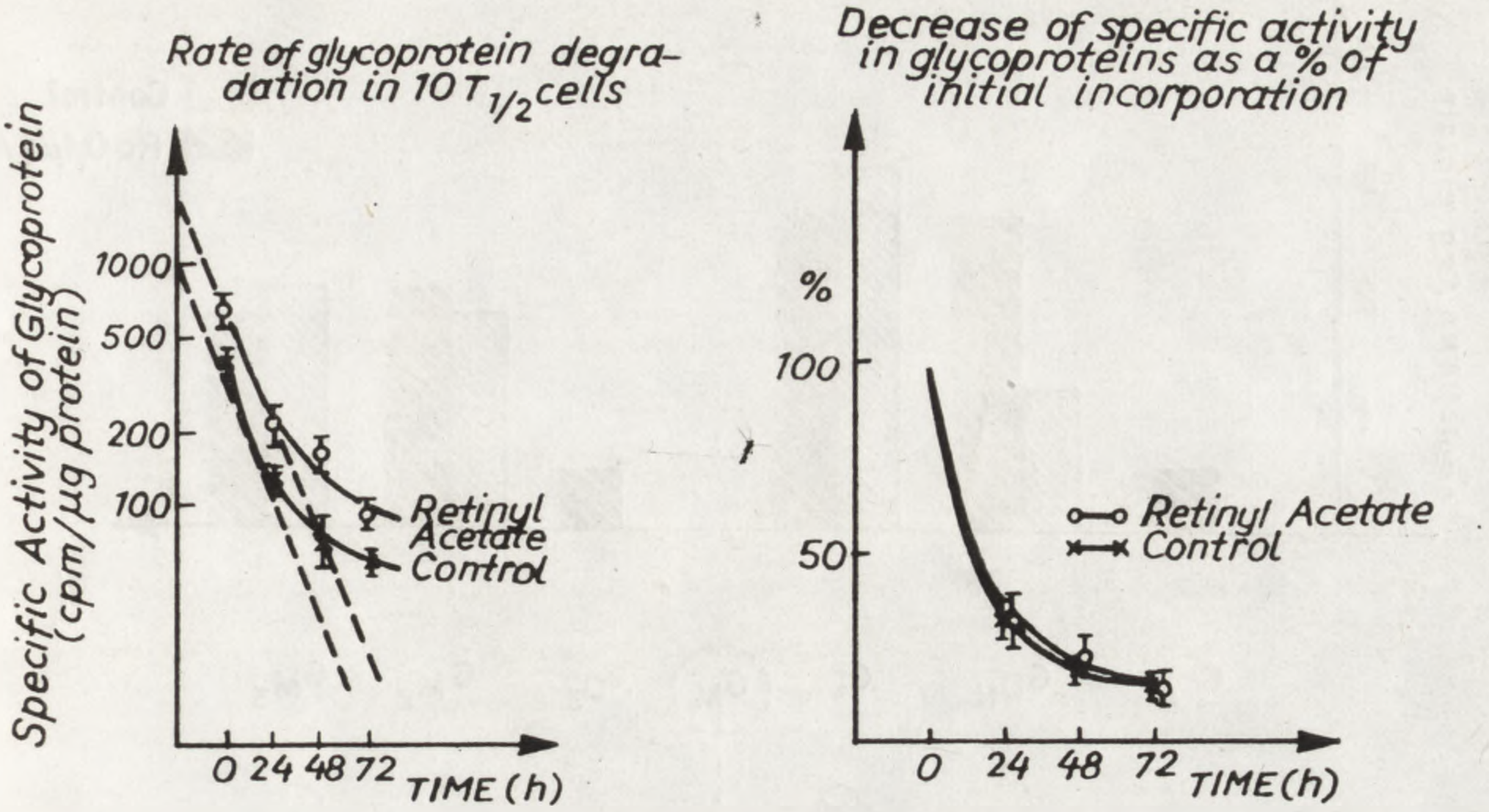

Fig. 4

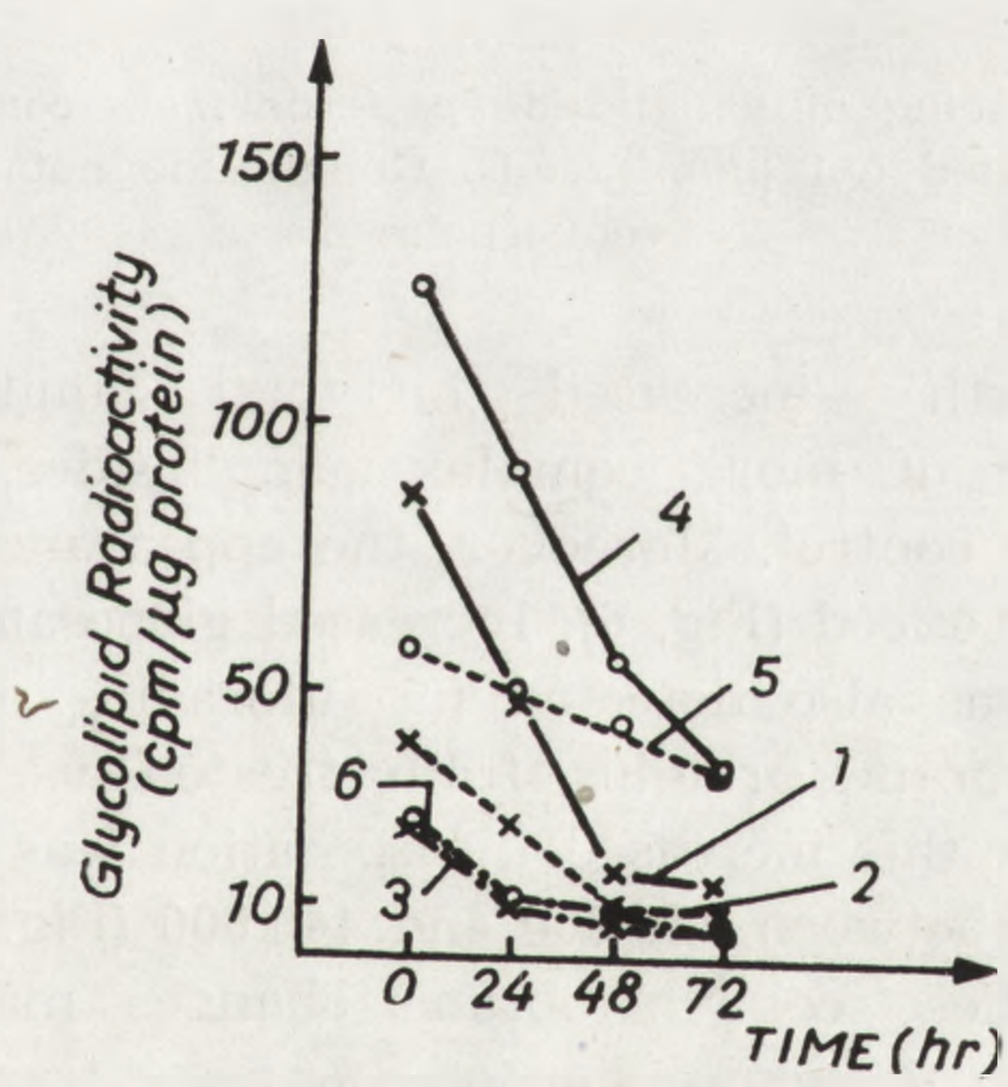

Fig. 5

Fig. 4 and 5. Degradation of glycoproteins and glycolipids in 10 $T_{1/2}$ fibroblasts grown to confluence in the presence of 0.1 µg/ml retinyl acetate. Confluent cultures were labelled by ^{3}H-glucosamine 10 µCi/dish and after 24 hours the radioactive medium was washed out and decrease of radioactivity in protein and lipid fractions was estimated in parallel dishes at 24 hours intervals

Control: *1* — total lipid, *2* — ganglioside, *3* — neutral lipid; Rentinyl acetate: *4* — total lipid, *5* — ganglioside, *6* — neutral lipid

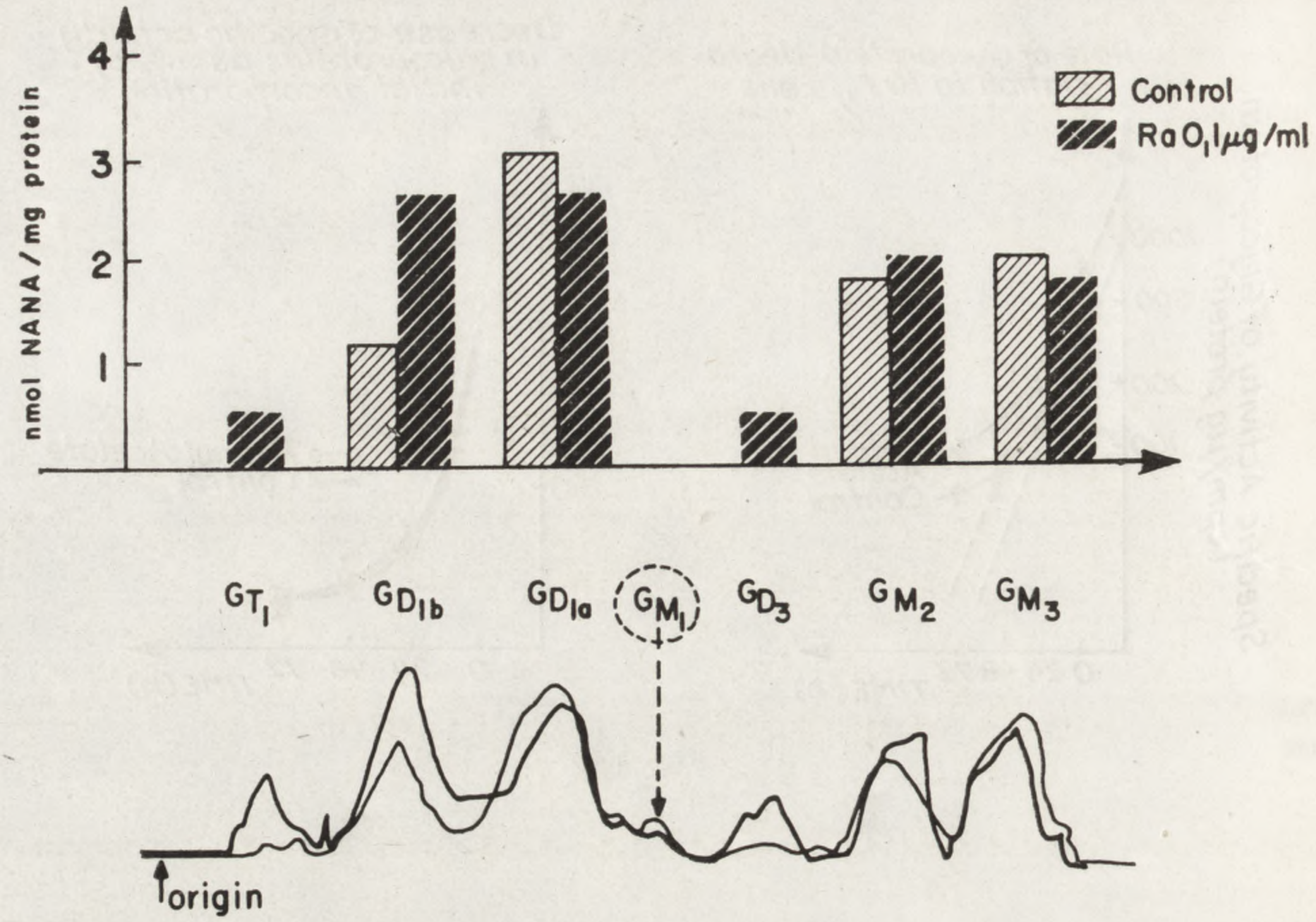

Fig. 6. Densitometer tracing of ganglioside patterns from control (light hatching) and retinyl acetate-treated (0.1 µg/ml) cells. Ganglioside notation is according to Svennerholm

rentinyl acetate further increased the total ganglioside concentration and proportion of more complex gangliosides, with G_{D1b} being increased 260% over control. Moreover, the appearance of G_{T1} and G_{D3} in treated cells was noticed (Fig. 6). Increased glucosamine incorporation into glycoproteins was also detected in autoradiographs of polyacrylamide gels of membrane proteins from treated vs. control cultures. Analysis showed that this increased incorporation was localized to large glycoproteins of Mw between 230 000 and 440 000 (Fig. 7). It is tempting to speculate that these cell membrane changes may effect cellular growth regulation and the expression of neoplastic transformation.

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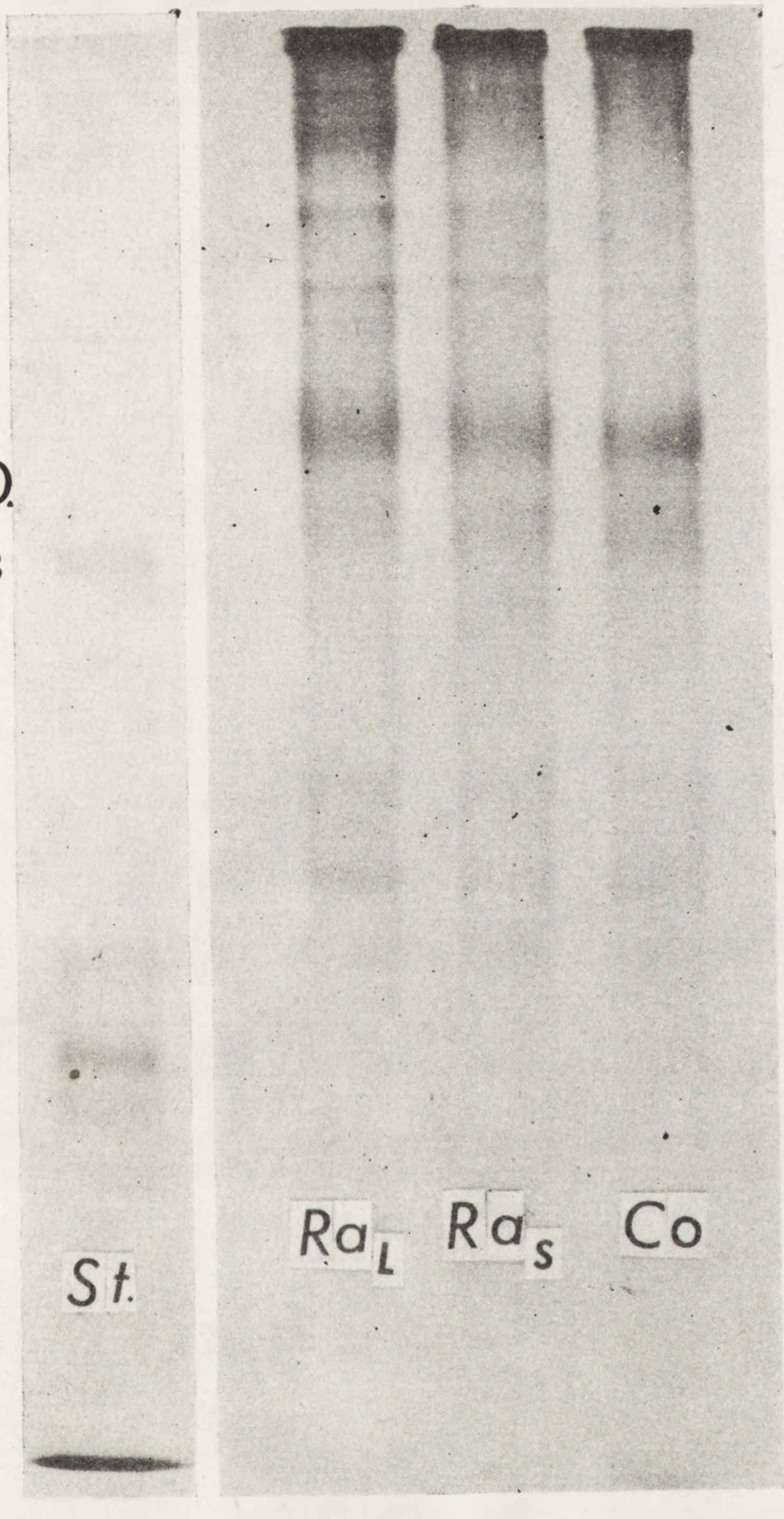

Fig. 7. Autoradiography of polyacrylamide gel electrophoresis of proteins isolated from 10 $T_{1/2}$ fibroblasts labelled 24 hours by ^{3}H-glucosamine (50 μCi/dish). Total radioactivity of proteins applied on gel was 40 000 cpm for each sample. 24 hours after seeding the cultures received 0.1 μg/ml of retinyl acetate (RA_L) or appropriate concentration of solvent followed by short (3 days) exposure to retinyl acetate (Ra_S) versus solvent only — treated controls (Co). All cultures were seeded into stated concentration of serum and grown to confluence (14 days). Left bar — coordinately run electrophoresis of standard proteins stained by coomassine blue

POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (645—648)

EXTRACELLULAR DNA AND ITS FUNCTION IN REGULATION OF LYMPHOCYTE PROLIFERATION *IN VITRO*

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In the course of the culture in vitro, lymphocytes release a part of their DNA into culture medium (reviewed in: [1]). A regulatory meaning of this phenomenon was hypothesized [2]. The present experiments were designed to study a correlation between lymphocyte proliferation in vitro and DNA concentration in the culture medium.

Human peripheral blood lymphocytes were cultured after mitogen stimulation in the presence of exogenous DNA. The following DNA preparations were alternatively used: human lymphocyte DNA, released DNA, calf thymus DNA, herring sperm DNA and mouse liver DNA. The first two preparations were isolated at the 4th day of the culture from PHA-stimulated lymphocytes and the culture medium, respectively.

Addition of DNA to the culture medium was followed by an inhibition of ^{3}H-thymidine incorporation to lymphocytes, at the peak of DNA synthesis, to 40–45% (lymphocyte DNA), 60–70% (released DNA) and 80–95% (foreign DNA) as compared to the control (Fig. 1). This effect was found to be time and dose dependent. DNA addition within first 48 hrs of the culture was prerequisite to evoke an inhibition of thymidine incorporation. The amount of exogenous DNA added to the medium ranged between $5–50 \times 10^{-6}$ g DNA per 10^6 cells, that is comparable with DNA content in cells, but is also 5–10 times higher than concentration of the released DNA. All the studied DNA molecules were of comparable size, except the released DNA (appr. 10^6–10^7 d). It was found, that the inhibitory effect of DNA was strongly dependent on its size. DNA degradation was parellelled with decrease of the

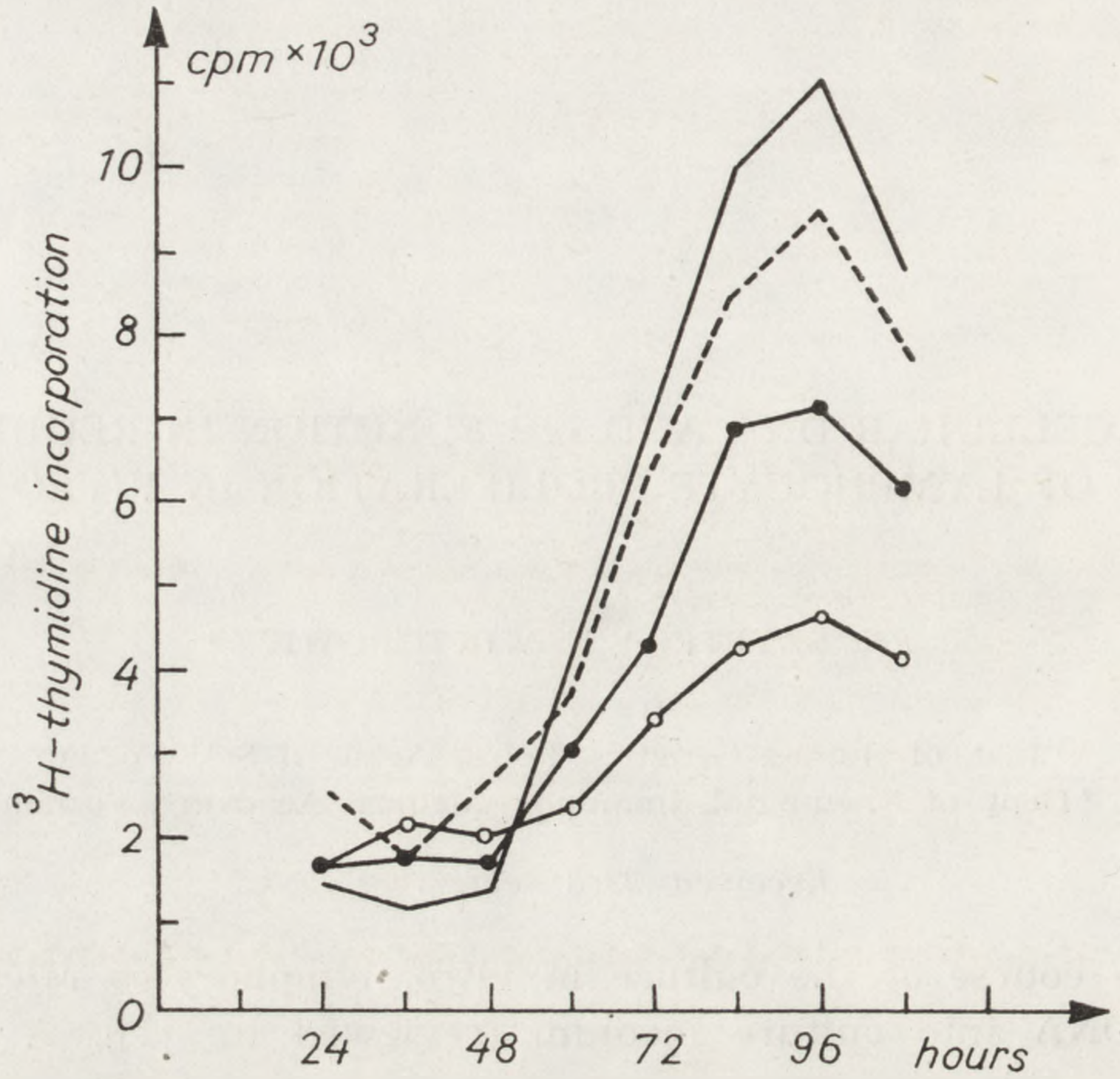

Fig. 1. ³H-thymidine incorporation to PHA-stimulated lymphocytes in standard conditions (solid line) and in the presence of lymphocyte DNA (open circles), released DNA (black circles) and calf thymus DNA (broken line)

observed effect. Incubation of lymphocytes with DNA in the absence of mitogen induced a low degree of stimulation (7–12% of blastogenesis).

An inhibition of ³H-thymidine incorporation was accompanied by a slight reduction of mitotic indices (Tab. 1). In this case we have also found exposition to homologous DNA to be more effective than to foreign DNA. No changes in the DNA cell cycle were observed.

TABLE 1

Mitotic indices found at different periods of standard culture and in the presence of added macromolecules

Specification	48 h	72 h	86 h	120 h
standard culture	0.0%	10.1%	6.0%	2.9%
+lymph. DNA (12 μg/ml)	0.2%	7.7%	3.6%	1.6%
+calf th. DNA (12 μg/ml)	0.2%	9.3%	5.6%	1.8%
+DNase I (20 μg/ml)	0.1%	11.2%	6.7%	3.4%

Although an exact mechanism of DNA interaction with proliferating cells is not known, we are able to exclude at least two possibilities. The inhibition of proliferation does not appear as a result of extracellular DNA-mitogen interaction. It has been shown by experiments with different mitogens, and by addition of DNA to PHA-free medium. Next, non-specific complexing of labelled thymidine to the extracellular DNA was also ruled out.

The presented results indicate that an increase of concentration of exogenous DNA does not affect the cell cycle of lymphocytes, but rather alters the number of proliferating cells. In the former paper [3], a significant increase of lymphocyte proliferation induced by DNase I was described. Altogether, there was obtained a good deal of evidence, supporting the idea on regulation of lymphocyte proliferation in vitro by homeostatic equilibrium between DNA concentration in cells and in the culture medium.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (649—652)

EFFECT OF THE AGENTS ELEVATING INTRACELLULAR CONCENTRATION OF CYCLIC NUCLEOTIDE, ON THE PROLIFERATION OF HUMAN LYMPHOCYTES

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Cyclic nucleotides seem to play an important role in the activation of lymphocytes to proliferation [3]. In the study presented here, we have investigated the suppressive action of theophylline (TF) and aminophylline (AF), two phosphodiesterase inhibitors, on the proliferation of mitogen-stimulated human peripheral blood lymphocytes.

Mononuclear cells were isolated from heparinized human peripheral blood by sedimentation on Ficoll/Uropolinum gradient ($d=1.077$ g/cm^3) [1]. For proliferation assay, 1×10^5 cells per well were cultured on flat-bottom microplates (Falcon, USA) in total volume of 0.2 ml of Eagle's medium supplemented with 10% of autologous plasma or 0.5% of human serum albumin. Streptomycin (100 μg/ml) and penicillin (100 IU/ml) were added to the culture medium as the preservatives. In some experiments, amethopterin (Methotrexate, Lederle), was added to a culture medium at a final concentration of 2.2×10^{-7}M [6]. All cell cultures

TABLE 1

Effect of aminophylline (AF) on the proliferation of PHA-stimulated lymphocytes (a representative experiment). Lymphocytes were cultured 3 days in Eagle's medium supplemented with 10% of autologous plasma. AF was added 24 hrs after beginning of the culture, to a final concentration 10^{-4} M

Reagent added to the lymphocyte culture	Total number of cells in microculture after 3 days (in 10^3 cells)	% of lymphoblasts in the 3 days cultures	Incorporation of ^{3}H-Tdr in 10^3 cpm
none	164.1	92	94.4
AF	95.6	81	66.9

TABLE 2

Effect of aminophylline (AF) on the ^{3}H-Td^r incorporation by PHA-stimulated lymphocytes (a representative experiment). Lymphocytes were cultured 4 days in Eagle's medium supplemented with 0.5% human serum albumin. Incorporation of ^{3}H-Tdr into AF treated lymphocytes was expressed as the percent of ^{3}H-Tdr incorporation into lymphocytes in control cultures

Day of addition of AF to the cultures	Concentration of AF in 10^{-4} M				
	25	12.5	6.25	3.12	0
0	2	15	60	81	100
1	15	37	42	62	100
2	15	33	70	67	100
3	29	46	73	102	100

were performed in triplicate. Phytohaemagglutinin (PHA, HA 17, Wellcome, 1 μg/ml) and concanavalin A (Con A, Pharmacia, 5 μg/ml) were used as mitogens. Theophylline (Sigma) or aminophylline (Polfa) served as the agents, increasing intracellular level of cyclic AMP (cAMP). Cells were cultured for 3–4 days at 37°C in a humidified atmosphere containing 5% CO_2. 2 hrs before harvest 1 μCi ^{3}H-Tdr (UVVVR, CSSR) per well was added. Cells were collected on glass fibre filters and washed 3 times with water. Radioactivity was estimated in a liquid scintillation

TABLE 3

Effect of theophylline (TF) and aminophylline (AF) on the ^{3}H-Tdr incorporation by lymphocytes stimulated with PHA or Con A (a representative experiment). Lymphocytes were cultured 3 days in Eagle's medium supplemented with 0.5% human serum albumin and 2.2×10^{-7} M of amethopterin. TF or AF were added to a final concentration 5×10^{-4} M. Incorporation of ^{3}H-Tdr into TF or AF treated lymphocytes was expressed as the percent of ^{3}H-Tdr incorporation into lymphocytes in control cultures

Day of addition of reagent to the cultures	Reagent	Mitogen used for stimulation of lymphocytes	
		PHA	Con A
0	none	100	100
	TF	75	60
	AF	35	25
1	TF	82	78
	AF	42	55
2	TF	77	103
	AF	90	58

counter. Percent of lymphoblasts was calculated from cytosmears, stained with May-Grünwald-Giemsa. For estimation of total number of cells in the culture, the known number of formalin fixed chicken erythrocytes was added to each microculture well, before matching smears by the Shandon cytocentrifuge.

AF and TF caused significant inhibition of lymphocyte proliferation, measured by ^{3}H-Tdr incorporation, percent of lymphoblasts and total number of cells, when added in milimolar concentration to the human blood lymphocytes cultured in vitro (Tab. 1). This effect was dose-dependent (Tab. 2). AF inhibited lymphocyte proliferation stronger than TF at equal concentration (Tab. 3). The maximum of inhibition was observed, when AF and TF were added at the beginning of the lymphocyte culture (Tab. 2 and 3). Similar results were obtained in experiments, where amethopterin, preventing lmphocyte switch to phase S of cell cycle, has been used (Tab. 3). The inhibitory effect depended on the kind of mitogen used (Tab. 3).

The results of this study are in good agreement with those of Hirschhorn et al. [2] and Smith et al. [5], concerning inhibitory effects of elevated intralymphocytic concentration of cAMP upon lymphocyte proliferation. Inhibition of proliferation caused by AF and TF was better marked, when these agents were added at the beginning of the cell culture, rather than later. This allows us to suggest, that they influence the very early events in cell activation. Observation on the inhibition of lymphocyte activation in the cultures, where cells were blocked in phase G_1 of the cell cycle, support this suggestion.

The lymphocyte proliferation, induced by PHA was less senstitive to inhibition with AF or TF, than that induced by Con A. Similar results were obtained by Novogrodsky et al. [4]. The reason for such differences still remains unclear. Thus, because AF and TF are widely used in clinical practice, their immunosuppressive action deserves further investigation.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (653—656)

THE INTERACTION OF HUMAN LYMPHOCYTES WITH SPERMATOZOA

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The in vitro interaction among human peripheral blood lymphocytes and ejaculated spermatozoa was studied. The cells were co-cultivated in an autologous and allogeneic system. Stimulation of lymphocytes induced by spermatozoa was measured by estimation of tritiated thymidine incorporation after 3 and 6 days of culture. The ability of spermatozoa to elicit lymphocyte proliferation in both the culture systems studied was shown [5]. The intensity of lymphocyte proliferation increased up to the 8th day of culture. The proliferation was higher in the allogeneic system than in the autologous one, when ejaculated spermatozoa were used as stimulators [5, 6]. Spermatozoa obtained from spermatocoele (cyst of epidydymis or testis) induced more intensive reaction in autologous combination [7]. Evaluation of surface markers on the lymphocytes after culture with spermatozoa, applying the E rosette test of Jondal for identification of T cells [4], and the immunofluorescent method of Frøland for B cells [2], revealed stimulation of heterogenous population of lymphocytes i.e. both T and B cells (Table 1).

TABLE 1

Results of E rosette and IF tests on lymphocytes from 6-day cultures with different levels of proliferation induced by spermatozoa. Studies were performed using 10 lymphocyte donors and fresh sperm samples from 4 individuals

Tests applied	Control lymphocytes	Allogeneic system	Autologous system	Control lymphocytes	Allogeneic system	Autologous system
Stimulation index	—	45.2±3.2	25.4±6.2	—	4.2±1.2	2.2±1.3
E rosette	45.6±8.4	79.7±6.4	75.2±6.4	52.6±4.3	38.6±6.6	40.3±7.2
IF test	7.2±1.3	13.4±0.5	12.2±1.7	7.4±1.1	6.5±1.4	5.3±1.2

TABLE 2

Blast transformation of lymphocytes induced by spermatozoa of infertile men. Results are given in stimulation indices (SI)

Donor of spermatozoa	Total number of spermatozoa in ejaculate	Percentage of spermatozoa with progressive motility	Percentage of spermatozoa with abnormal morphology	SI in Autologous	SI in Allogeneic cultures.
1	60×10^6	20	40	—	1.3; 1.4; 1.4
2	75×10^6	25	50	3.8	23.5
3	90×10^6	33	44	—	1.9; 2.1
4	64×10^6	42	60	3.7	2.2
5	54×10^6	24	34	16.0	20.1
6	120×10^6	50	35	—	1.5; 2.9
7	95×10^6	32	60	—	4.1; 1.5
8	105×10^6	30	42	1.2	1.0
9	94×10^6	25	30	—	1.2; 1.3
10	120×10^6	40	30	0.9	0.9
11	90×10^6	35	60	0.9	0.8
12	70×10^6	25	30	0.8	1.0
13	65×10^6	50	45	1.0	1.0

The percentage of spermatozoa with progressive motility did not correlate with the intensity of the spermatozoa-provoked lymphocyte proliferation [6]. The attempt to correlate proliferation level of lymphocytes and number of abnormal morphological features of spermatozoa was also unsuccesful (Table 2).

The proliferation of lymphocytes varied from individual to individual, and some samples of spermatozoa obtained from healthy individuals did not exert any stimulatory effect on lymphocytes from different donors [5].

Recently, the influence of acrosomal enzymes isolated from spermatozoa on lymphocyte proliferation was observed during the first three days of culture (Table 3).

The problem of presence [3] or absence [1] of HLA antigens on human spermatozoa, and their possible role in the lymphocyte-spermatozoa interaction studied, is highly controversial. The few experiments designed to detect these antigens by the immunoprecipitation method with monoclonal antibodies, revealed rather minimal quantities of HLA antigens on human spermatozoa. Hence, it seems that these surface antigens did not take part in lymphocyte-spermatozoa interaction. It could not be excluded, however, that lymphocyte proliferation induced by spermatozoa is elicited by spermatozoal antigens quite different from those of the main histocompatibility complex.

TABLE 3

Stimulation of autologous and allogeneic lymphocytes by acrosomal preparation in 3-day culture. The results are given as stimulation indices±SD. The results were obtained using spermatozoa from 10 individuals and lymphocytes from the same number of donors

Specification	Autologous system				Allogeneic system			
Acrosomal prep. (protein dose)	1.8 μg	3.6 μg	7.5 μg	15 μg	1.8 μg	3.6 μg	7.5 μg	15 μg
Stimulation Indices	1.0±0.3	1.7±0.4	2.8±0.6	1.4±0.4	1.1±0.3	1.9±0.4	3.0±0.5	1.3±0.3

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (657—660)

ULTRASTRUCTURAL INVESTIGATIONS OF GROWTH AND DIFFERENTIATION OF MAMMARY GLAND CELLS

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The results of histochemical investigations [1] were the main cause for undertaking ultrastructural studies of the growth and differentiation of mammary gland cells, and of the mutual relations of the processes of secretory cell differentiation in the production of morphologically determinable milk ingredients. Also, the probable function of myoepithelial cells in the differentiation process, was studied with special interest.

Mammary gland samples were taken from female Balb c mice during pregnancy and immediately after delivery. Tissue fragments were fixed in Franke's fixative [2], stained in lead citrate and uranyl acetate [3]. Thin sections were examined in a Tesla BS 500 electron microscope.

At the resting stage, mammary gland secretory cells have a poor cytoplasmic organization, lobated nuclei with regularly dispersed chromatin, and smal nucleoli. The cytoplasm, specially, has few ribosomes which are generally not connected with sparse ergastoplasm. The Golgi apparatus which is situated in the central part of the cell, consists of only a few lamellae and single vesicles of various size. The mitochondria are few in number and small, with widely spaced internal cristae. Small myoepithelial cells situated on the alveoli peripheries have a poor cytoplasmic organization, with only a few fibrillar structures. The first symptoms of secretory cell differentiation appear already in the middle of the first week of pregnancy. The mitochondria become larger and more numerous, with more densely arranged internal cristae. The ergastoplasm with ribosomes develops and simultaneously widens its cisternae. At the same time, the first fat secretion in the form of a few droplets of various size and arrangement, becomes visible in the cytoplasm (Fig. 1). Fat drops are most frequent in the supra- and paranuclear region. They can also be found in the base part of the cell, which may

Fig. 1. 3rd day of pregnancy. The first lipid drop (LD). N — nucleus, GA — Golgi apparatus, BL — basal lamina. Magnification: 12 000 ×

cause a dislocation of the nucleus. Fatty secretion is most characteristic in the cells situated in the vicinity of myoepithelial cells, where a fat drop of large diameter occupies nearly all the cytoplasm. All the forms of secretion have intermediate contact with the ergastoplasm and the mitochondria. Fat drops may be also observed in the lumen of some alveoli. At the turn of the first and second week of pregnancy, the already described changes in the ergastoplasm are being accompanied by changes in the Golgi apparatus which begins to move towards the apical part of the cell. The flattened cisternae become more numerous, and the vesicles increase in size and number, especially in the peripheral part of the apparatus. The first few protein granules become visible in the vesicles (Fig. 2). They are either flocculent and of high electron density or appear as clearly separated round granules of various size. No clear influence of the vicinity of secretory cells to myoepithelial cells on the process of protein granule formation could be observed. The ergastoplasm is at that time very well developed. As the cells differentiate towards the production of both kinds of secretion, their nuclei grow larger, become round in shape, and move towards the basement membrane. The concentrations of chromatin surround the nucleus membrane peripherally, and the nucleoli get larger.

It should, however, be pointed out, that not until the second half of

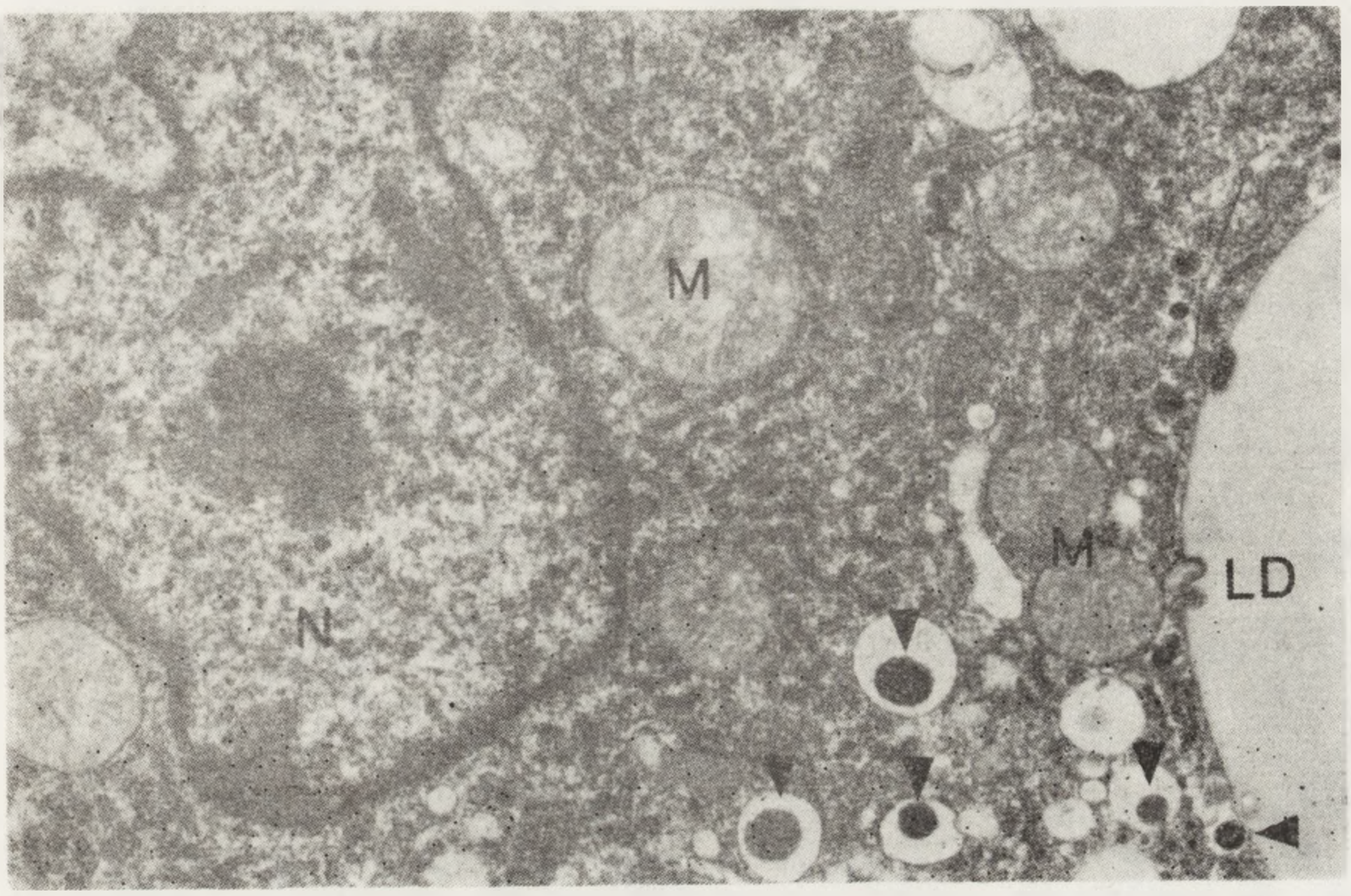

Fig. 2. 7th day of pregnancy. Protein granules (arrows). M — mitochondria, N — nucleus. Magnification: 12 000 ×

pregnancy do myoepithelial cells acquire the stage of differentiation close to final.

The further stages of differentiation that occur in the second half of pregnancy and at the beginning of lactation, have been dealt with in other papers [4, 5].

Conclusions:

1. Secretory cells differentiate towards the production of MFG faster than towards the production of protein secretion.

2. Myoepithelial cells participate mainly in the process of the forming of fatty secretion.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (661—664)

GROWTH RESPONSE OF *TETRAHYMENA PYRIFORMIS* GL TO SALTS ADDED AND TO DEPTH OF CULTURE

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Cameron [1], Hill [2], and Levy [3] pointed out that the rate of gaseous exchange (which might be altered for example by depth of the culture) and some differences in the composition of the medium are extracellular, regulatory, or turning factors for aerobic and/or anaerobic pathways of the cellular metabolism in *Tetrahymena.* Thus, the relatively modifiable quality and quantity of end-products of energy metabolism, liberated by cells into the culture medium, will modify chemical conditions of cultivation. Rate of cell multiplication and intensity of cellular growth, both affecting growth of the culture, could be influenced by end-products of metabolism. Thus, dynamics of growth of non-aerated culture in limited volume of medium of different composition, could be labile. The effects of end-products of cellular metabolism on culture growth have not always been considered, however different culture conditions are applied in experiments with *Tetrahymena.* Differences in culture growth dynamics, as a presumable effect of different levels of culture depth, combined with the effects of optimal and suboptimal trophic conditions, will be presented in this paper.

Tetrahymena pyriformis GL, the amicronucleate strain of ciliated protozoan, was investigated. Cells were cultivated axenically in Erlenmayer's flasks of equal shape and content (200 ml), and in optimal temperature (28°C) without aeration. Two kinds of culture medium were used: "PPY" — containing 1.5% proteosopepton (DIFCO), and 0.1% yeast extract (DIFCO), as well as "PPY + S" — additionally supplemented with mineral salts (Mg^{+2}, Fe^{+2}, Mn^{+2}, Zn^{+2}, Ca^{+2}, Cu^{+2}) according to Plesner et al. [4]. Initial levels of depths of culture medium were estimated as 0.53, 1.11, 1.74, 2.45 cm when their volumes were 25, 50, 75 and 100 ml respectively. Experimental cultures were inoculated from stock culture exponentially growing in "PPY+S", and obtained initial densities were 3×10^3 cells/ml. Cultures were sampled after every 12 or 24 hrs. using sterile technique

and cells were counted electronically. Rates of cell multiplication during consecutive phases of growth and duration of these periods, levels of maximal densities reached in cultures and duration of stationary phase, and finally, rates of cell number decline, were compared.

Different courses of culture dynamics were obtained (Fig. 1). Effects of culture medium depths were more coherent and more distinct in "PPY+S" than in "PPY". Rates of cell multiplication were discriminative (Fig. 2). During the exponential phase of growth, only the shallow cultures in "PPY+S" (0.53 cm) showed a high rate of multiplication (doubling time 2.5–3.0 h). The curves of dynamics of cell numbers in cultures were generally lowered with the increase of culture depth (Fig. 3). The character of differences between effects of depth in "PPY+S" and "PPY" cultures suggests that acidous end-products of energy metabolism could be important limiting factors influencing the dynamics of culture growth. This assumption seems to be supported by the results of the pH estimations, especially in "PPY+S" cultures (Fig. 4), and meoreover by comparison of stationary phase delay and by related

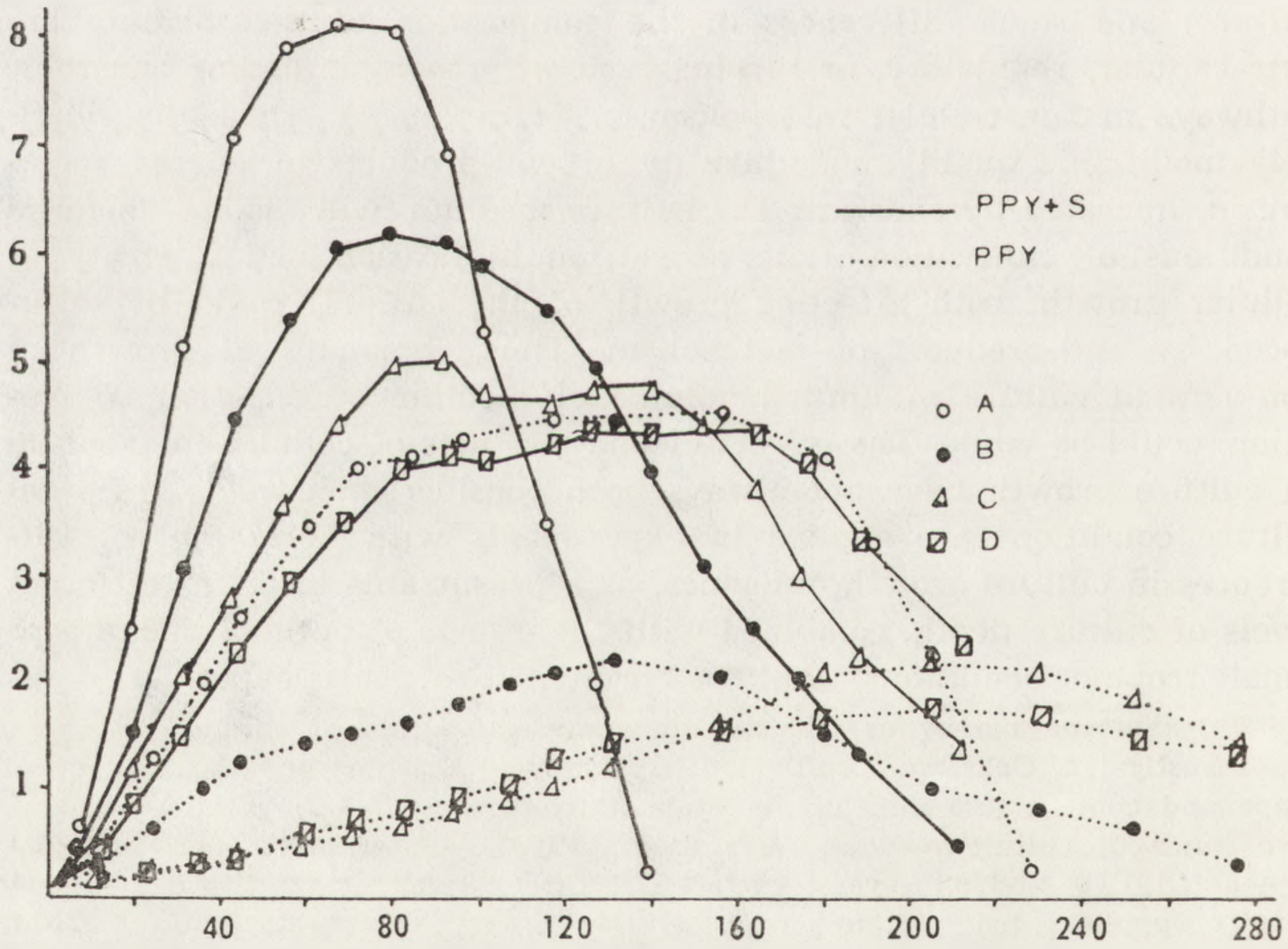

Fig. 1. Dynamices of growth of *Tetrahymena pyriformis* GL as the effects of medium ("PPY+S" and "PPY") and different depths of culture. Depths of culture: *A* — 0.53 cm, *B* — 1.11 cm, *C* — 1.74 cm, *D* — 2.45 cm. Abscissa: time in hours. Ordinate: cell density in cultures × 10^5/ml

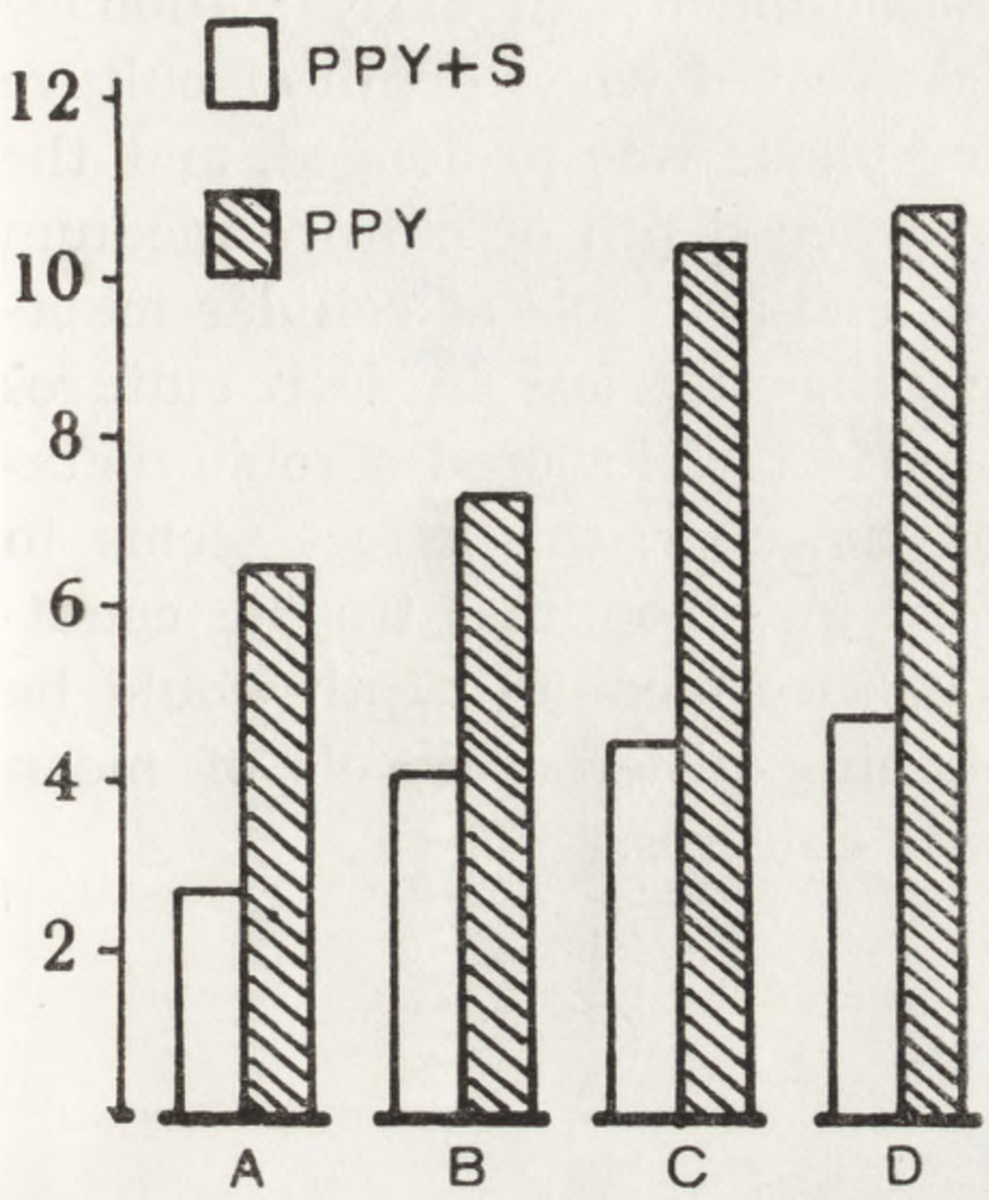

Fig. 2. Rates of doubling of cell numbers during exponential growth in experimental cultures. Depths of culture: *A* — 0.53 cm, *B* — 1.11 cm, *C* — 1.74 cm, *D* — 2.45 cm. Ordinate: doubling time in hours

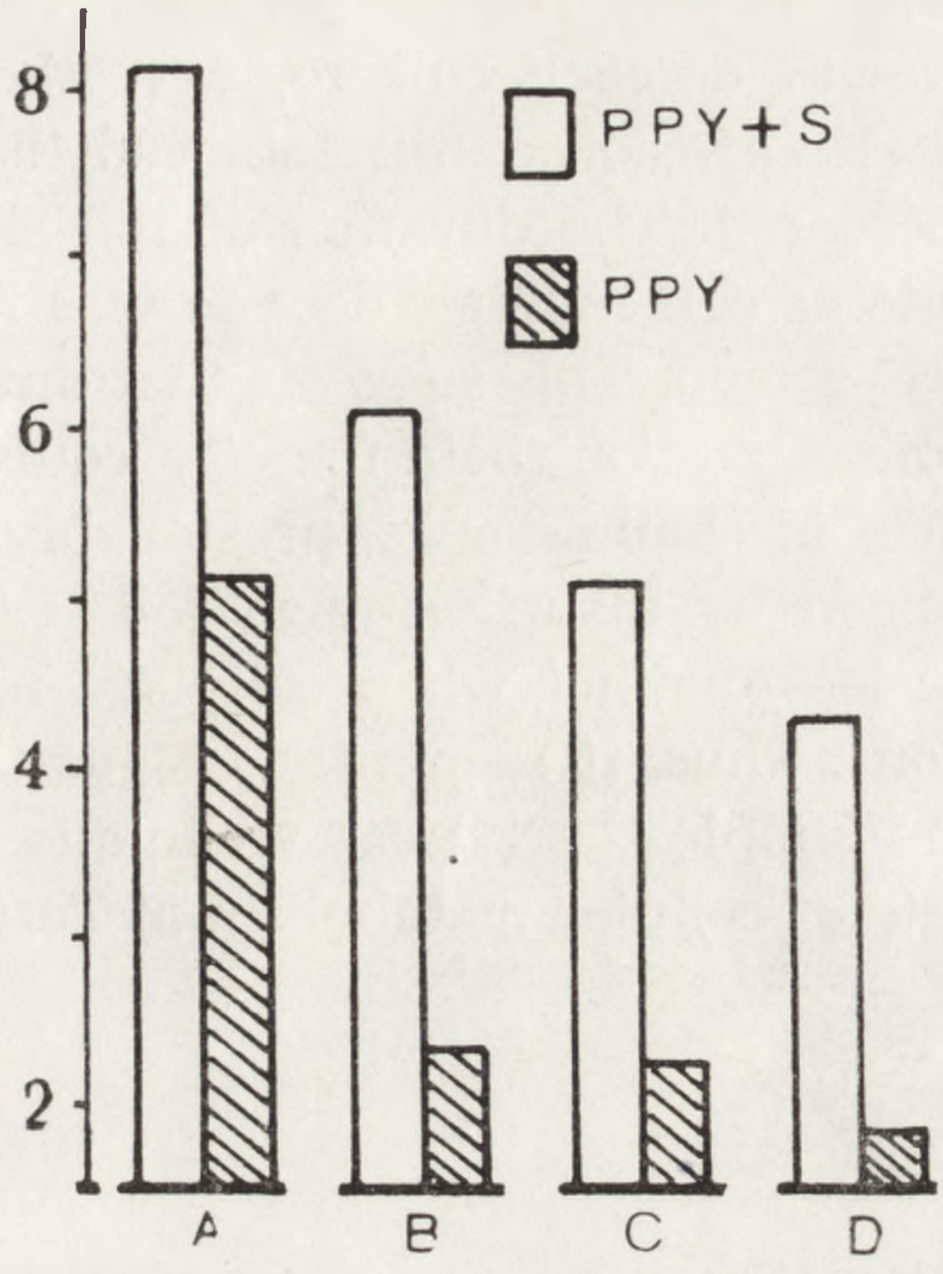

Fig. 3. Maximum cell densities in stationary phase of experimental cultures. Depths of culture: *A* — 0.53 cm, *B* — 1.11 cm, *C* — 1.74 cm, *D* — 2.45 cm. Ordinate: cell number × 10^5

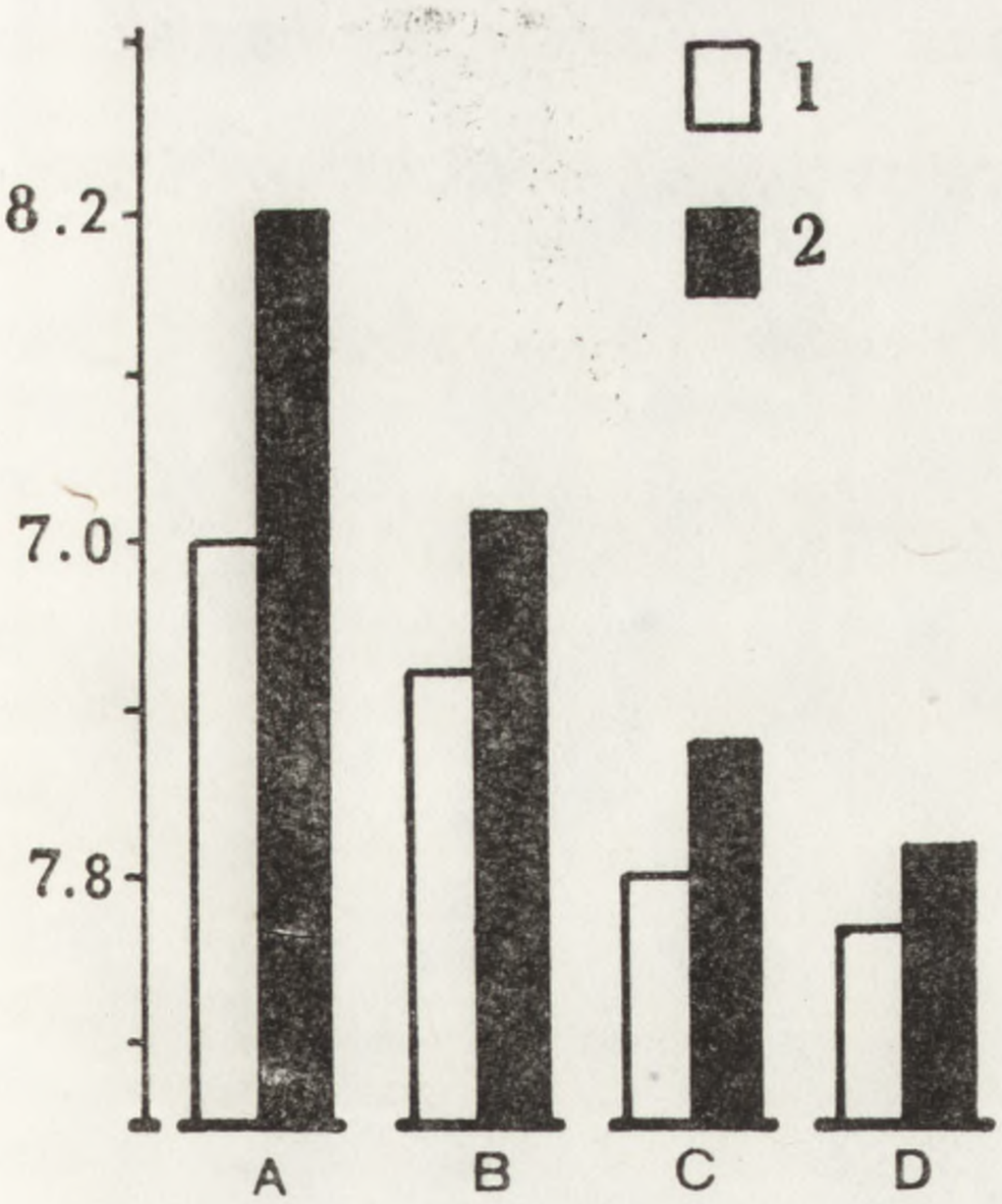

Fig. 4. Effects of pH estimation in culture medium sampled from early stationary cultures in "PPY+S" medium. Measurements were carried out in centrifuged and filtered (cell free) samples (*1*) and repeated after incubation (15 minutes) in boiling water bath (*2*) . Depths of culture: *A* — 0.53 cm, *B* — 1.11 cm, *C* — 1.74 cm, *D* — 2.45 cm. Oridinate: values of pH

rates of cell number decline. Values of pH generally increased during growth of each culture, nevertheless measurements in early stationary phases of each culture showed that pH decreased when depth of culture increased. The duration of the stationary phase was prolonged, and the rate of cell number decline was lowered when depth of culture medium was grown. This suggests that amounts of end-products of cellular metabolism and/or their toxicity could be significantly less in deep cultures than in shallow ones, presumably as the effect of lowered aerobic metabolism by insufficient gaseous exchange; however, this effect seems to be less distinct when cells are maintained in suboptimal trophic conditions. Thus, it seems that regulation of culture medium depth would be the simplest practical technique in obtaining different levels of mean rate of cellular metabolism in *Tetrahymena* cultures.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (665—668)

LOW-MOLECULAR PRODUCTS OF AUTOLYSIS OF CANCER CELLS AND THEIR NUCLEI

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In recent years, there have been plenty of articles about the action of endogenous low-molecular factors upon cell growth and proliferation. Hirschfeld, Blumenthal and other authors, pioneers of cancer research, published now-forgotten papers reporting about the clinical and experimental good effects of cancer therapy with autolyste from cancer tissue [1]. Some experimental papers published recently have again been dealing with the problems of inhibitory factors isolated from cancer tissue [2–5].

The idea behind the present paper is the assumption that some kind of inhibitory factor is formed during the natural autodigestion of cancer cells, when the proteins are being cut by means of natural composition of endogenous enzymes. It has also been assumed that the inhibitory factors are formed as a result of autodigestion in the cancer cell nucleus.

Ehrlich ascites tumour (EAT) cells were obtained from 30 male, suffering mice by peritoneal puncture. Then the cells were washed in Phosphate Buffered Saline (PBS) 37°C and resuspended to density 5×10^5 cells/ml. Such a suspension was incubated 72 hours in 37°C. After that, the suspension was centrifuged 1400 × g and supernatant was lyophilised. This procedure was repeated 30 times with each mouse separately. After each stage the fluids were tested microbiologically with negative effects. Then the lyophilisates were put together, dissolved in 100 ml of distilled water and dialysed 24 hours in 4°C into 1000 ml of distilled water. The distilled water to which low-molecular products of autolysis diffused through semipermeable membrane was lyophilised, whereas the dialytic bag with its contents was removed. The lyophilisate was dissolved in 150 µl of distilled water and fractionated using thin-

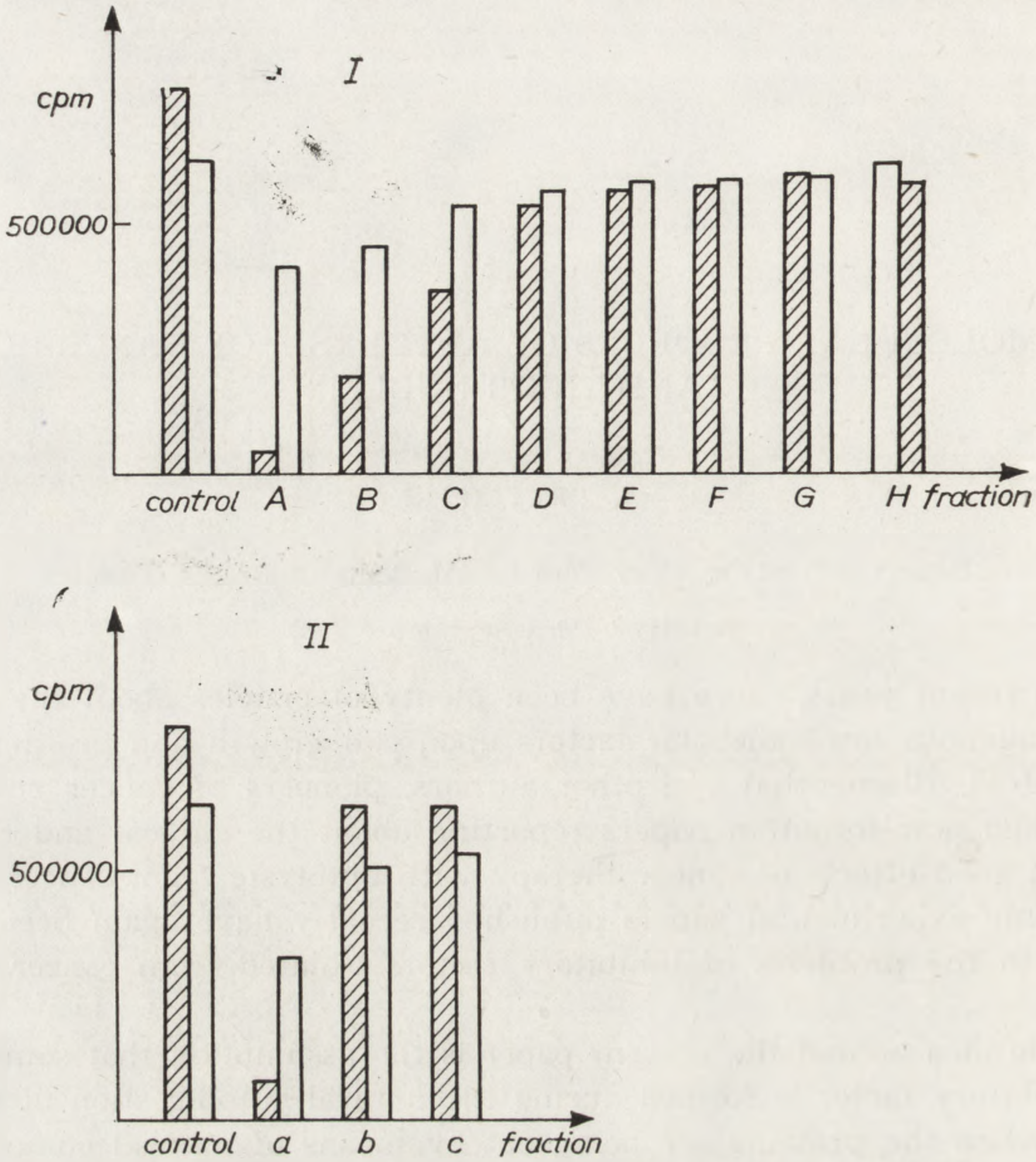
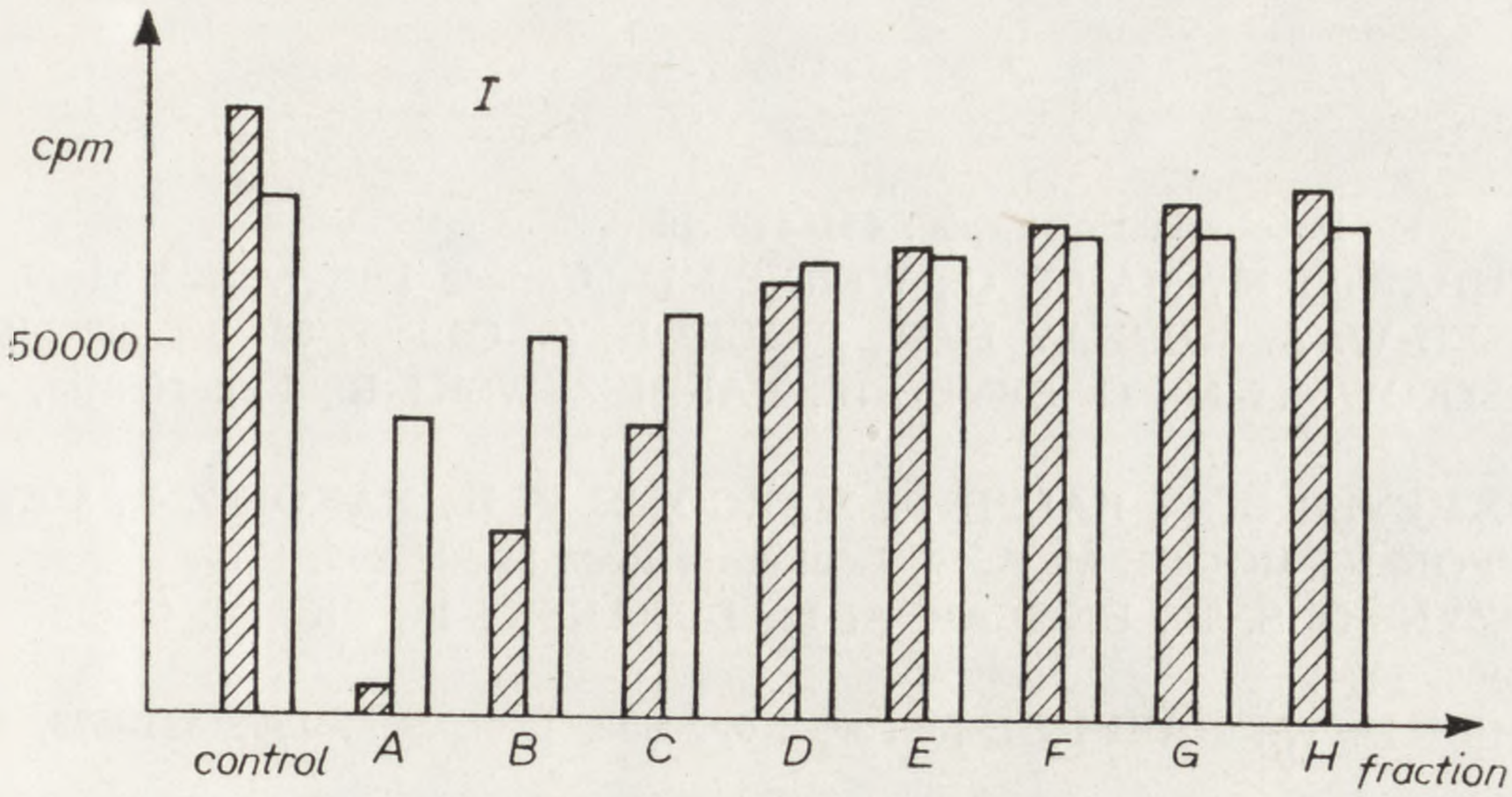


Fig. 1. Incorporation of ^{3}H-Thymidine to EAT cells (■) and to normal mice thymocytes (□) treated with (1 μg/ml) low-molecular products of autolysis of EAT cells (I) and low-molecular products of autolysis of nuclei isolated from EAT cells (II) fractionated by thin layer chromatography on Silicagel G

layer chromatography method as described by Burzynski [6]. The obtained fractions were eluted and tested for their effects in suspension of EAT cells and in the suspension of thymocytes from healthy mice. Testing was carried out by the method of incorporation of ^{3}H-Thymidine and ^{3}H-Uridine. The experiment was repeated again but this time the inhibitory factor was obtained from the nuclei isolated from EAT cells using Fisher's method [7] with Tween 80. Isolated nuclei were treated with the procedure described above.

The results of the experiment are given in Figs. 1 and 2. In the first series 8 fractions were obtained. All the fractions have inhibited ^{3}H-Thymidine and ^{3}H-Uridine incorporation. Fraction A was the most active



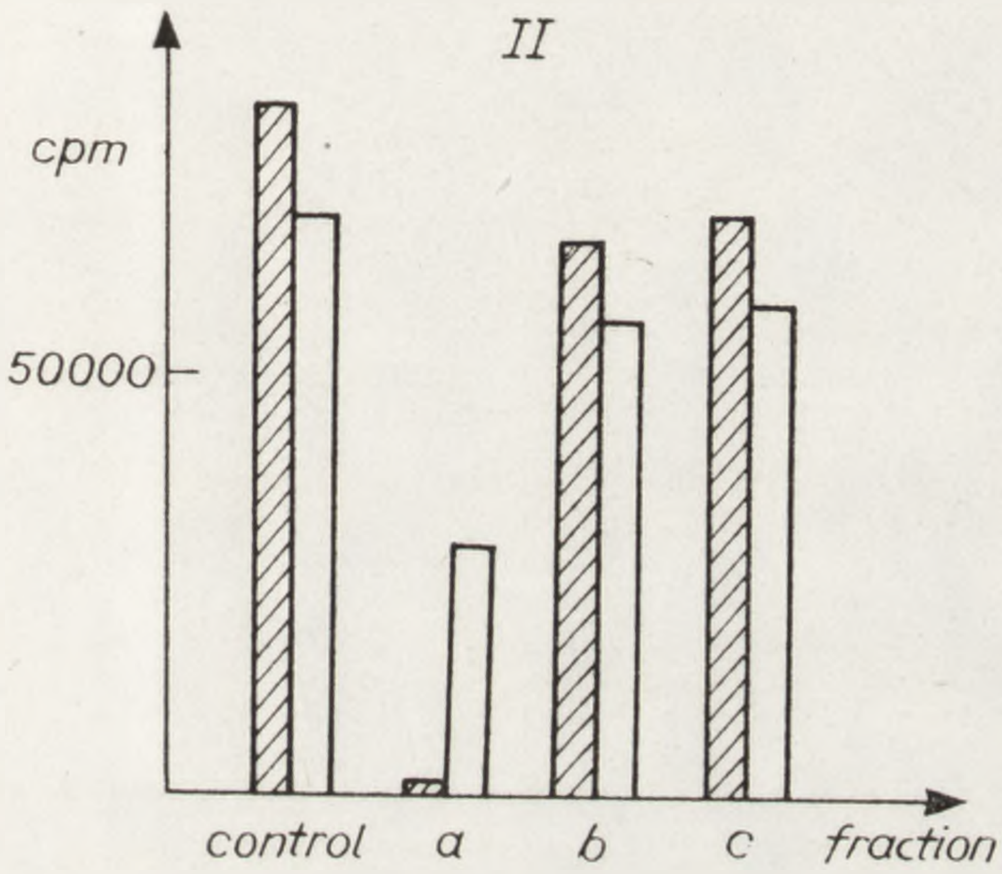


Fig. 2. Incorporation of ^{3}H-Uridine to EAT cells (■) and to normal mice thymocytes (□) treated with (1 μg/ml) low-molecular products of autolysis of EAT cells (I) and low-molecular products of autolysis of nuclei isolated from EAT cells (II) fractionated by thin layer chromatography on Silicagel G

one (95% of inhibition). In the second series, due to autodestruction of nuclei, 3 fractions were obtained. The most active one was fraction a (98% of inhibition).

The inhibitory factors obtained in the experiments may be products of the degradation of the nucleoproteins. The degradation of the nucleoproteins, caused by the presence of active catheptic enzymes in the nuclei, occurs during the incubation of the cancer cells and the nuclei isolated from them.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (669—672)

AGGREGATES OF L 1210 LEUKAEMIA CELLS DURING THEIR GROWTH IN VITRO

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Lymphatic leukaemia cells L 1210 provide a useful model for studying the metabolism of neoplasmic cells, while their behaviour when cultivated may supply some informations concerning of their functioning in vivo. Experiments have been carried out in order to elucidate the phenomenon of aggregates formation by leukaemic cells, the description of the conditions for aggregate formation, and their disaggregation into single cells.

Two lines of lymphatic leukaemia cells L 1210, which primarily grew in vitro for 90 and 400 days, respectively, obtained from the Laboratory of Cytophysiology Medical Centre of Postgraduate Education, were used. The cells from both primary cultures were cultivated for 9 days in Eagle'a medium with 10% calf serum, antibiotics and 0.5%

TABLE 1

The results of measurement of cell density, percentage of dead cells and cell diameter

Day	Cell number/ml × 10_5		Percent of dead cells		Cell diameter μm	
	90 d line	400 d line	90 d line	400 d line	90 d line	400 d line
0	3.0±0.4	1.99±0.2	12±3.0	13±3.0	—	11.6
1	3.2±0.3	2.7±0.3	17±5.0	11±2.0	—	13.5
2	7.1±1.7	5.8±1.0	11±2.0	8±1.0	10.6	12.2
3	7.2±0.7	7.4±0.6	11±2.0	29±4.0	11.1	11.5
4	8.9±1.2	6.6±0.6	44±6.0	47±3.0	10.6	10.95
5	8.9±0.9	6.7±0.3	45±6.0	74±2.0	10.7	11.1
7	11.0±1.6	6.8±0.6	75±3.0	84±13.5	10.7	10.6
9	10.2	6.95	83.0	91.0	9.3	9.99

N-2-Hydroxyethylpiperazine-N-2 ethane sulfonic acid (HEPES), pH 7,3–7,4. The following parameters of culture were controlled every day: number of single and aggregated cells, percentage of living cells and cell diameter. In order to establish the nature of substance, the cells suspensions of cell aggregates were treated with the following enzymes: trypsin (Serva), DNA-ase I (Sigma), RNA-ase ex bovine pancreas 5× cryst. (Koch-Light), elastase pancreas 2× crystallized (Sigma), at concentrations 0,1 or 0,05%. The levels of lipids, nucleotides, nucleic acids, DNA and proteins were estimated by biochemical methods and read spectrophotometrically with a VSU-2 (C. Zeiss Jena) spectrophotometr. A or B version of Kołmogorow-Smirnow's nonparametrical one-sides or two-sides test for the significance of differences, were used for verification results [1].

L 1210 lymphatic leukaemia cells displayed varying density increases during the 9 days of observation. The greatest increase of density of the 400-day-old population occurred on the third day of cultivation, and did not change from that moment. In the 90-day-old population, 3 peaks of cell density increase occurred: on the 2–3rd, on the 4–5th and on the 7th day. It could provide grounds for claiming the existence of the not-uniform cell population. The viality of cells in both populations did not differ significantly. The diameter of cells within an observation did not change, either. Aggregates formation was estimated in 400-day-old populations only. The number of aggregates increased up to the third day of cultivation, and then it decreased up to the 6th day, and went up again

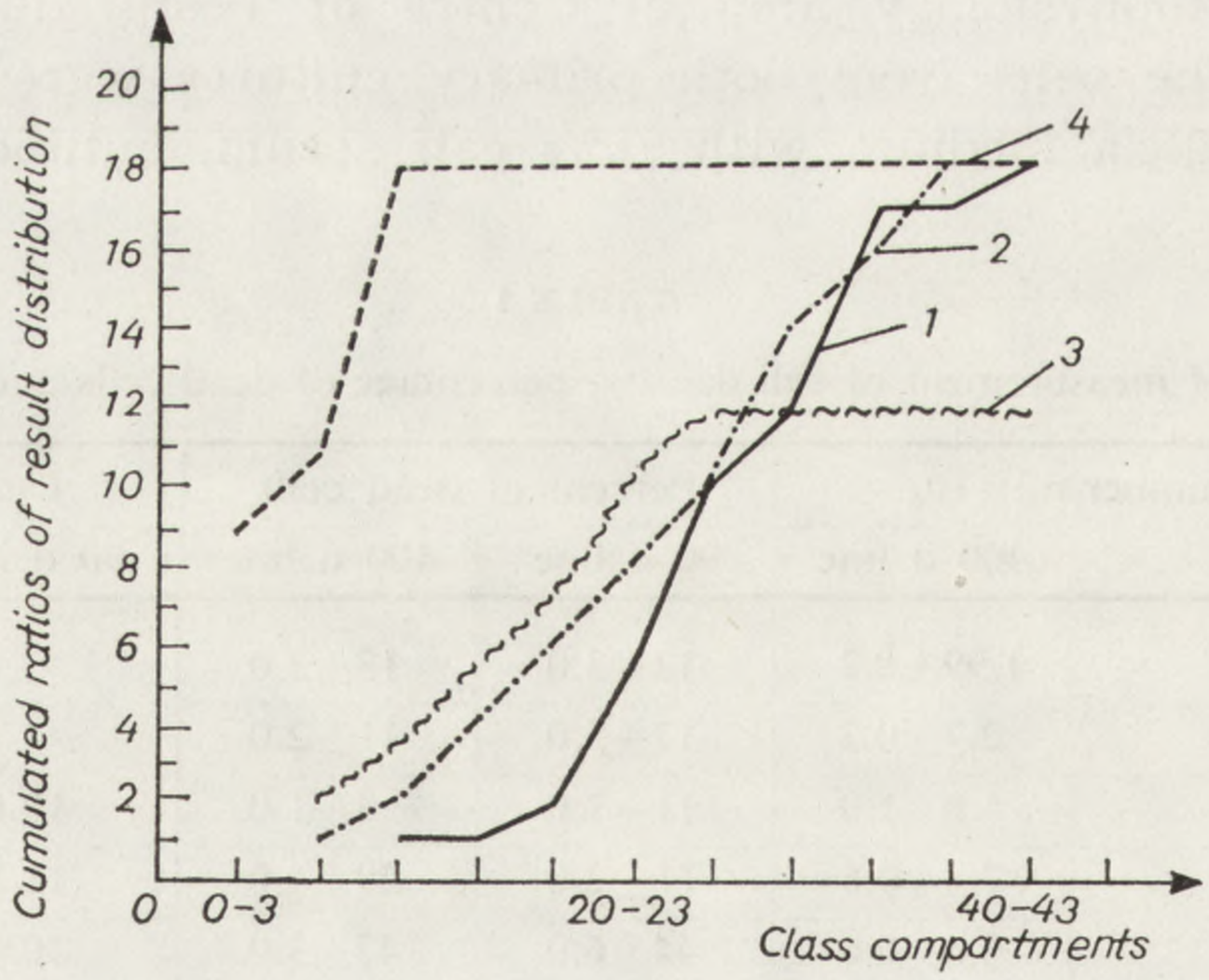

Fig. 1. The percentage of cells in aggregates after treatment of cultures with enzymes; *1* — DNA-aza, *2* — RNA-aza *3* — Elastase, *4* — Trypsin

at the end of the observation period. On the first and second day there appeared aggregates consisting of living cells only, and they consistuted almost half (47%) the total of the aggregates. The remaining aggregates consisted of both living and dead cells. Starting on the 5th day of cultivation, the aggregates consisting of living cells were absent, while dead cell aggregates appeared just on the 4th day and their percentage kept increasing with time. When three-day-old cultures had been treated with enzymes, only trypsin caused disaggregation and digestion of dead cells which percentage was decreasing with respect to the control culture. This result may be indicate that cells are cemented into aggregates by some protein material. In vivo trombine, or some other coagulation factors from IX-XIII, may play a similar role as trypsin in vitro, and prevent of aggregate formation [2–4]. The level of nucleic acids and protein, which is the highest at the beginning of culture, indicated and intensified bolism of cultivated cells in that period.

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POSTĘPY BIOLOGII KOMÓRKI TOM 11, NR 3—4, 1984 (673—674)

THE EFFECT OF LUNG-CONDITIONED MEDIA ON TUMOR CELL PROLIFERATION IN VITRO

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Received: 23rd September 1983

Several growth-promoting factors were obtained from tumors as well as from normal tissue [1–3]. In the present work we have been looking for tumor growth modifying substances in lungs. Their existence was suggested by works on animal systems. Conditioned media (CM) were obtained by 48 hr incubation of small pieces of lungs in serum free medium. The media were then separated by dialysis. The obtained non-dializable material called D-1 stimulates the growth of neuroblastoma N2a cells, as can be judged by plating efficiency method. The dializable part (D-2) in turn inhibited proliferation of tested cells. The results presents Table 1. It is worth mentioning that media were also tested

TABLE 1

Effect of conditioned medium on plating efficiency of N2a cells

Control	19.7 ± 4.2 *	12.2 ± 3.2 **
D-1	27.0 ± 1.1	17.5 ± 3.0
D-2	11.2 ± 4.7	7.2 ± 2.3

Mean value of 5 dishes. * — 100 cells seeded.
** — 60 cells seeded.

against human mesothelial umbilical cells, human mammary tumors (MCF), and human epithelial cells (HEp). All examined cells showed similar patterns of reaction on CM as neuroblastoma cells. In the next step, CM were separated by gel filtration on Sephadex G-75. The results showed that the stimulatory factor was eluted in fractions having relative molecular mass between 40 000–70 000. The inhibitory activity was found in fractions between 12 000–20 000 and below 5000. In order to

TABLE 2

Effect of proteaze treatment on D-2 activity

Control	D-1	D-2	D-2 digested
32.7 ± 3.7	44.7 ± 3.8	20.5 ± 4.5	33.2 ± 4.9

Plating efficiency — mean of 5 dishes. 100 cells seeded.

exclude the non-peptide nature of inhibitory substances, the dializable fraction of CM (D-2) was digested with protease. The treatment abolished inhibitory effect (Table 2). From the preliminary studies, it looks as if inhibitory factor/s have an affinity with gangliosides. This has been documented by short term incubation of D-2 medium with non-inhibitory content of gangliosides (Fidia, Italy) [4].

TABLE 3

Effect of gangliosides (G) on plating efficiency of N2a cells and D-2 activity

Control	G(1.00 mg/dish)	D-2	D-2 + G
67.5 ± 13.5	64.0 ± 5.6	25.0 ±4.2	42.3 ± 4.0

Mean of 5 dishes. 200 cells seeded.

Such treatment reduces the inhibitory effect of the medium. These results are presented in Table 3. The presence of two kinds of growth regulating substances in lungs, can suggest a local regulatory mechanism. Its knowledge may be of importance for the treatment of tumor micrometastasis.

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SPIS TREŚCI

Warszawa, listopad 1984 r.

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