

25 °C as necessary, and examined before and after post-fixation in formaldehyde vapour. The preparative and examination procedures were essentially the same as those described in earlier papers<sup>9-11</sup>, except for the ionic composition and osmolarity of the BSS. Since the observed differences between cells processed in different ways were trifling, a single description will suffice.

The level of lactate dehydrogenase activity in erythrocytes and their precursors was several orders of magnitude lower than that in leucocytes and thrombocytes in the same preparations, or in other types of cells<sup>9-11</sup>. In erythroblasts and early erythrocytes, demonstrable lactate dehydrogenase activity is largely confined to mitochondria. The mitochondria are numerous, filamentous, and often aligned end-to-end; most are found within a perinuclear region of the cytoplasm, sometimes forming a skein round the nucleus, more often sticking out radially toward the periphery of the cell. The remainder of the cell, including nucleus, endoplasmic reticulum, compound and simple globules, remains completely unstained. As erythrocytes begin to mature, and microspectrophotometrically detectable haemoglobin accumulates in the cytoplasm, the staining pattern gradually changes (Figure 1). The number of mitochondria decreases, and they become shorter; as haemoglobin accumulates, the mitochondria become packed into a narrow juxta-nuclear zone. Now, in addition to the mitochondria, a few cytoplasmic globules also show lactate dehydrogenase activity. Nuclear lactate dehydrogenase activity, both diffuse and associated with condensed chromatin, begins to appear. As maturation reaches completion, the nuclear lactate dehydrogenase activity increases (Figure 2). Often, not more than 2 or 3 active mitochondria remain in the cytoplasm. A few cytoplasmic globules are still positive, but the greater part of the total lactate dehydrogenase activity of the mature erythrocytes become nuclear. There are large variations in the amount and pattern of lactate dehydrogenase activity between individual erythrocytes.

In view of the generally low rate of enzymatic activity in erythrocytes, it was necessary to use suitable controls to exclude the possibility that the observed staining pattern might be due to unspecific effects rather than to the distribution pattern of lactate dehydrogenase activity. The staining was attributable to enzymatic activity, since the cells were completely unstained when lactate was omitted from the incubation medium. The tetrazolium salt captured electrons at the site of enzymatic activity, and not beyond the flavoprotein, since the localization pattern was quite different when the incubation medium contained NADH<sub>2</sub> and cytochrome *c* or menadione, in-

stead of lactate and NAD<sup>+</sup>. Finally, lactate dehydrogenase and not some other NAD-linked glycolytic enzyme contributed the electrons, since added pyruvate substantially blocked the staining reaction<sup>1</sup>.

We conclude from these experiments that there is an unequivocal negative correlation between the level of nuclear lactate dehydrogenase activity and the degree of clear genetic activity of DNA in *Amphiuma* red blood cells. Another cell in which this correlation seems to be valid is the mesocerebral neurone of the snail, *Helix pomatia*, in which nuclear lactate dehydrogenase activity had also been found<sup>10-11</sup>. Activation of lactate dehydrogenase by DNA had been noted briefly by MAZIA<sup>12</sup>. Several hypotheses arise out of the present findings: 1. lactate dehydrogenase may be involved in the regulation of DNA transcription; 2. in addition to any possible relationship between DNA and lactate dehydrogenase, the nucleus of the mature amphibian erythrocyte may show a form of 'adaptive metabolism', i.e., function in terms of intermediary metabolism as though it were a piece of cytoplasm. These hypotheses are being examined systematically in our laboratory.

**Résumé.** Dans les erythroblastes et jeunes hématies de la salamandre géante, *Amphiuma tridactylum*, l'activité de la deshydrogénase lactique n'est localisée que dans le chondriome. Pendant l'accumulation de l'hémoglobine et au cours de la croissance des hématies, l'activité devient de plus en plus nucléaire. On constate une corrélation négative entre l'activité de la deshydrogénase lactique nucléaire et l'activité génétique de l'ADN.

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<sup>12</sup> D. MAZIA, in *The Cell* (Eds. J. BRACHET and A. MIRSKY; Academic Press, New York 1960), vol. III.

<sup>13</sup> This work was carried out whilst one of us (A.M.A.) was in receipt of an undergraduate scholarship from the Boettcher Foundation, tenable at the College of Arts and Sciences, University of Denver; support from the Undergraduate Scholars' Program of this College is also gratefully acknowledged. We are grateful to Dr. H. G. DAVIES (MRC Biophysics Research Unit, London) and Dr. S. M. MCGEE-RUSSELL (State University of New York, Albany) for criticism of the manuscript.

## Morphactins, Growth and Auxin Transport

Biological activity of morphactins – a new group of plant regulators<sup>1</sup> – was first reported by SCHNEIDER<sup>2</sup> on a few of them. These chemicals cause striking biological changes over an extraordinarily broad range<sup>3</sup>. Few works have been devoted before now to compare various synthesized morphactins<sup>3</sup>. In the present investigation, parallel action of 4 morphactins on plant cell growth and the in vitro auxin translocation<sup>4</sup> will be analyzed, using epicotyl segments of *Lens culinaris*.

Seeds were first soaked for 6 h in deionized water, washed and placed in terralite (dark; 22 °C). When used

for experimental purposes, after 96 h, the length of the epicotyls was approximately 14 mm. For the *growth experiments*, 8.0 mm segments were placed (20 per 4 ml) in a buffered solution (pH 6.1) with 2% sucrose on a shaking

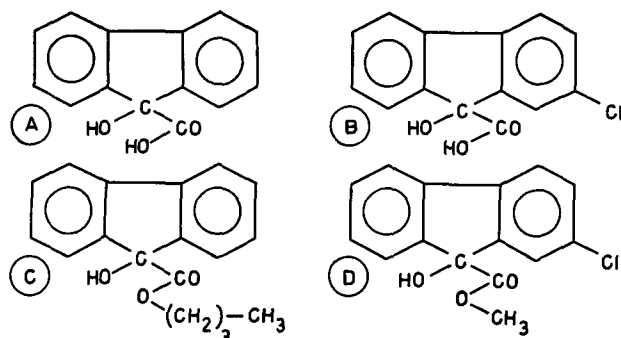
<sup>1</sup> G. MOHR, Dt. Bot. Ges. Symp. 3, 5 (1969).

<sup>2</sup> G. SCHNEIDER, Naturwissenschaften 51, 416 (1964).

<sup>3</sup> G. SCHNEIDER, Dt. Bot. Ges. Symp. 3, 19 (1969).

<sup>4</sup> P. E. PILET, Int. Conf. Plant Growth Regul., Carleton (1968), p. 993.

incubator (dark;  $25 \pm 0.5^\circ$ ) for 12 h<sup>5</sup>. For the *auxin transport experiments*, 6.6 mm segments were immersed<sup>6</sup> in a Ringer solution containing (or not) morphactins (50  $\mu\text{g}/\text{ml}$ ). After 2 h incubation, segments were placed, for 5 h, in a horizontal position. An agar block (donor) incorporating  $\beta$ -indolylacetic acid (IAA-2-<sup>14</sup>C; sp. act. 13.3 mC/mM; conc.  $3 \times 10^{-5} M$ ) was applied to one cut section; the auxin transport is estimated by measuring the radioactivity



- A) 9-hydroxyfluorene-(9)-carboxylic acid (IT 3235).  
 B) 2-chloro-9-hydroxyfluorene-(9)-carboxylic acid (IT 3299).  
 C) *n*-butyl-9-hydroxyfluorene-(9)-carboxylate (IT 3233).  
 D) methyl-2-chloro-9-hydroxyfluorene-(9)-carboxylate (IT 3456).

Table I. Effect of 4 morphactins on the relative elongation of the 8.0 mm sections prepared from the etiolated *Lens* epicotyls.

Concentration (M)	Morphactins			
	IT 3235	IT 3299	IT 3233	IT 3456
$10^{-8}$	5	6	16	41
$10^{-7}$	12	17	27	65
$10^{-6}$	19	19	34	66

Incubation time: 12 h; 20 sections per 4 ml of buffered solution (pH 6.1) with 2% sucrose (dark;  $25 \pm 0.5^\circ\text{C}$ ). Results in % of the control (stimulation); each value, average of 80 sections.

Table II. Effect of a morphactin's pretreatment on the in vitro basipetal transport of the IAA-2-<sup>14</sup>C

	Ringer	Ringer + Morphactins			
		IT 3235	IT 3299	IT 3233	IT 3456
Donors					
$D_0$	9858	9726	9706	9725	9891
$D_5$	5028	5826	6018	6419	7221
Loss from donors					
$\Delta D = D_0 - D_5$	4830	3904	3688	3306	2670
Receivers					
$R_5$	368	265	225	205	107
Relative intensity ( $R_5/\Delta D$ ) $\times 10^3$	75	67	61	62	40

Diffusion time: 5 h. Pretreatment: 2 h. 40 sections for 25 ml of a Ringer solution and morphactin (50  $\mu\text{g}/\text{ml}$ ). IAA-2-<sup>14</sup>C in donor blocks ( $3 \times 10^{-5} M$ ); spec. act. 13.3 mC/mM. Radioactivity in DPM per block. Each result, average of 40 blocks.

appearing in the block (receiver) placed at the other end of the segment<sup>7</sup>. It has been previously observed, for a similar material, that there is a predominantly basipetal movement of <sup>14</sup>C from IAA-2-<sup>14</sup>C<sup>8</sup>. Therefore, only this transport will be tested here. Analyses of <sup>14</sup>C were performed in a Nuclear Chicago Mark 1 liquid scintillation computer Model 6860<sup>9</sup>; results were expressed in terms of disintegrations (DPM) per block<sup>10</sup>.

Four morphactins – kindly supplied by E. Merck AG, Darmstadt – were tested.

As regards the growth of the organ segments, only IT 3456 was previously tested on the *Triticum* coleoptiles<sup>11</sup> and the *Avena* mesocotyls<sup>12</sup>. As can be seen in Table I, a) all the morphactins used slightly increased the cell elongation; b) IT 3456 is the most active morphactin, then, in order of decreasing action, IT 3233, IT 3299 and IT 3235.

It was already noted, for wheat, pea and sunflower<sup>13</sup> and for pea<sup>14</sup>, that IT 3456 may dislocate the endogenous auxin movement. It was therefore interesting to analyze, for the 4 morphactins used here, the kinetic of the IAA-2-<sup>14</sup>C transport (5 h) and the several processes associated with the in vitro IAA translocation<sup>4</sup>. It can be observed in Table II that the morphactins tested significantly reduce the in vitro transport of IAA: a) the IAA uptake (loss from donors)<sup>8</sup> was decreased and IT 3456 was found to be the most active, IT 3235 the least; b) the relative intensity<sup>15</sup> of the IAA movement (radioactivity in receiver per unit of uptake) was also reduced and, here again, the action of IT 3456 was strongest while that of IT 3235 was weakest.

In conclusion, the stimulation of the elongation of the epicotyl segments and the inhibition of the IAA-2-<sup>14</sup>C translocation were clearly observed for the 4 morphactins tested. The 2 parallel processes are surely related to the chemical structure since, for both elongation and IAA-transport, IT 3456 was the most active and, in decreasing degree, IT 3233, IT 3299 and IT 3235. These facts certainly explain the typical action of the new compounds on the growth of plant cells.

**Résumé.** Les morphactines stimulent l'allongement de segments d'épicotyle de *Lens*, inhibent l'absorption de l'IAA-2-<sup>14</sup>C et réduisent l'intensité du transport in vitro de cette hormone de croissance. Ces propriétés, liées à la structure chimique de ces composés (par ordre d'activité croissante: IT 3235, IT 3299, IT 3233 et IT 3456) permettent de rendre compte de leurs effets sur l'auxèsis des cellules végétales.

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<sup>12</sup> H. HARADA, *Dt. Bot. Ges. Symp.* 3, 77 (1969).

<sup>13</sup> E. KRELLE and E. LIBBERT, *Planta* 80, 317 (1968).

<sup>14</sup> F. TOGNONI and A. ALPI, *Dt. Bot. Ges. Symp.* 3, 53 (1969).

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