radiation was used for the experiments on cell survival, and lower levels for those on chloroplast formation. During the irradiation, the cells were continuously agitated in M/25 KH_2PO_4 solution in a petri dish. Cells were withdrawn at varying time intervals and plated on agar medium. Procedures were carried out under a dim red light and the plates were incubated in the dark for 6 days when the colonies were visible. When the fraction of green colonies was counted, the 6-day plates were exposed to light for further 3 days or more to permit the full development of chloroplasts.

For LHR experiments, UV-irradiated cells were held in M/25 KH_2PO_4 solution for 4 days in the dark before plating. The results are shown in figure 1. The fraction of cells surviving was clearly increased by this procedure (figure 1, a). On the other hand, figure 1, b shows that liquid holding brought about no change in the sensitivity of chloroplast formation.

To see the effect of CAF, *Euglena* cells, after UV-irradiation, were spread on the plates containing CAF at 0.1%. This concentration of CAF was known to have no effect on cell survival or on cell bleaching in the control cells. Figure 2, a shows that fewer surving colonies were obtained on the CAF-containing plates.

But in contrast to this, no effect of CAF on chloroplast formation was observed (figure 2, b). The possibility of non-permeation of CAF into the chloroplast was excluded, since we had already observed that a certain number of bleached cells were always produced by 0.5% CAF. From these data, we assume that cell viability, which is controlled by the nuclear system, contains a repair mechanism. On the other hand, the chloroplasts of *Euglena* lack this mechanism. Therefore, we conclude that this apparent lack of dark recovery of chloroplast formation is one of the main reasons why the *Euglena* chloroplast is so sensitive to UV-irradiation.

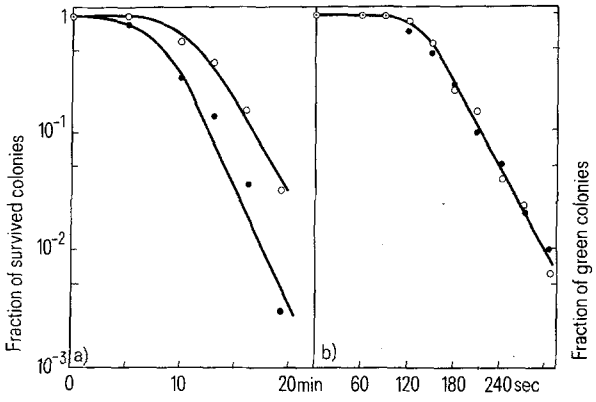

Fig. 2. Effect of caffeine on the colony formation after UV-irradiation. *a* Fraction of surviving colonies (15 W germicidal lamp); *b* fraction of green colonies (6 W germicidal lamp). ○ Without added CAF; ● with 0.1% CAF.

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Inhibition of *Dugesia tigrina* auricle regeneration by inhibitors of polyamine synthesis

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Summary. Results are presented that indicate polyamine synthesis inhibitors increase the flatworm *Dugesia tigrina*'s auricle regeneration time. This study serves as evidence that endogenous putrescine, spermidine, and spermine are necessary for the flatworm regeneration process.

Recently, Forbes et al.[1] presented evidence that putrescine and the polyamines, spermidine and spermine, are involved in the flatworm regeneration process by reporting data indicating that exogenously applied putrescine and the polyamines stimulate auricle regeneration in *Dugesia tigrina*. Further observations showing an involvement of these amines in flatworm regeneration are given in the present report. We applied inhibitors of polyamine synthesis to decapitated specimens of *Dugesia tigrina* and observed a significant increase in auricle regeneration time. This growth inhibition was prevented if putrescine, spermidine, or spermine was applied along with either of the inhibitors used. These results indicate that endogenous or naturally-produced putrescine and the polyamines are needed for the flatworm regeneration process.

The inhibitors used were methylglyoxal Bis(guanylhydrasone)(MGBG) and α-methylornithine (α-MO). MGBG is an inhibitor of putrescine dependent S-adenosyl-L-methionine decarboxylase[2] and therefore blocks spermidine and spermine formation[3]. α-MO is a competitive inhibitor of ornithine decarboxylase and thus inhibits the production of putrescine as well as the polyamines[4].

Materials and methods. 2 similar experiments were conducted (tables 1 and 2). Specimens of *Dugesia tigrina* were fasted 5 days in order to eliminate intestinal contents that might cause infection. Then for each experiment several animals were randomly assigned to a control group or to 1 of 7 treatment groups. Each animal was then anesthetized for decapitation by being placed on a filter paper presoaked with saline solution (Betchaku's solution[5] without Neomycin sulfate was used in this study) mounted on a petri dish filled with ice and then the animal's head was cut off immediatly behind the auricles. After decapitation, control animals were each placed in 25 ml of saline solution, while animals of the treatment groups were each placed in 25 ml of saline solution containing an amine (putrescine, sper-

Table 1. The effect of methylglyoxal Bis(guanylhydrazone) (MGBG) with and without putrescine, spermidine, or spermine on *Dugesia tigrina* auricle regeneration

Treatment	Number of animals	Mean time for auricle regeneration ± SD (days)
Control	20	6.20 ± 0.83
Putrescine (1×10^{-4} M)	20	$5.40 \pm 0.72^{a,b}$
Spermidine (1×10^{-4} M)	20	$5.05 \pm 0.67^{a,b}$
Spermine (1×10^{-4} M)	20	5.70 ± 0.58^{b}
MGBG (5×10^{-4} M)	17	8.85 ± 1.61^{a}
Putrescine (1×10^{-4} M) + MGBG (5×10^{-4} M)	17	$6.80 \pm 0.90^{a,b}$
Spermidine (1×10^{-4} M) + MGBG (5×10^{-4} M)	20	5.95 ± 1.05^{b}
Spermine (1×10^{-4} M) + MGBG (5×10^{-4} M)	18	6.00 ± 0.76^{b}

[a] $p < 0.01$ vs control; [b] $p < 0.01$ vs MGBG (5×10^{-4} M) treatment.

Table 2. The effect of α-methylornithine (α-MO) with and without putrescine, spermidine, and spermine on *Dugesia tigrina* auricle regeneration

Treatment	Number of animals	Mean time for auricle regeneration ± SD (days)
Control	15	6.33 ± 1.08
Putrescine (1×10^{-4} M)	15	$5.13 \pm 0.35^{a,b}$
Spermidine (1×10^{-4} M)	15	$4.93 \pm 0.25^{a,b}$
Spermine (1×10^{-4} M)	15	$5.00 \pm 0.00^{a,b}$
α-MO (5×10^{-4} M)	15	8.60 ± 1.12^{a}
Putrescine (1×10^{-4} M) + α-MO (5×10^{-4} M)	15	$5.06 \pm 0.25^{a,b}$
Spermidine (1×10^{-4} M) + α-MO (5×10^{-4} M)	14	$5.21 \pm 0.50^{a,b}$
Spermine (1×10^{-4} M) + α-MO (5×10^{-4} M)	15	6.32 ± 0.42^{b}

[a] $p < 0.01$ vs control; [b] $p < 0.01$ vs α-MO (5×10^{-4} M) treatment.

midine, or spermine at a concentration of 1×10^{-4} M), an inhibitor (MGBG or α-MO at a concentration of 5×10^{-4} M), or one of the amines (1×10^{-4} M) plus one of the inhibitors (5×10^{-4} M). The animals were then observed daily for the reappearance of auricles.

Results. The results are summarized in tables 1 and 2. Both inhibitors, MGBG and α-MO, significantly increased the mean time needed for auricle regeneration (analysis of variance and Tukey's (a) test were used). In both experiments, each amine when used alone reduced the mean time needed for auricle regeneration and each was effective in preventing or reducing the regeneration retardation effect of both inhibitors.

Discussion. Rupniak and Paul[6] using MGBG arrested the growth of cultured rat embryo fibroblasts and upon adding spermidine or spermine observed a rapid resumption of cell proliferation. Using α-MO, Mamont et al.[4] inhibited proliferation of rat hepatoma cells in culture. Addition of putrescine, spermidine, or spermine resulted in an immediate resumption of cell proliferation. Our results parallel these reports and further support the idea that the amines studied are needed for cell proliferation processes in general.

Flatworms have been neglected as subjects of polyamine research. Yet they are inexpensive, easily maintained and manipulated in the laboratory, and exhibit a spectacular ability to regenerate. The authors hope that this report and the one by Forbes et al[1] will stimulate an interest in utilizing flatworms in studies of the role of polyamines in growth processes.

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Uptake of NO_2 by plants grown at different salinity levels

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Summary. It is demonstrated that the uptake of nitrogen dioxide (NO_2) by *Phaseolus vulgaris* L. is decreased by the addition of sodium chloride (NaCl) to the root medium, as a result of increased diffusive resistance of the leaves. The NO_2-uptake rate constant measured kinetically was in agreement with the nitrite content of the leaves after the fumigation.

The uptake of gaseous pollutants by plants is a well known fact. Knowledge about the absorption of air pollutants under various environmental conditions is important with regard to the function of the plants as a sink. Several studies were carried out to demonstrate how the rate of absorption is related to the stomatal resistance[1,2]. It is generally accepted that stomatal resistance is inversely proportional to the molecular diffusivity of the gas of interest. The stomatal resistance of the leaves depends on such factors as the type, age and condition of the plant; the latter aspect is influenced by the water availability, solar radiation level, air temperature, concentration of CO_2 in the atmosphere, etc., as well as soil properties and damage by pollutants[3]. Under controlled environmental conditions the uptake of NO_2 has been thoroughly studied from a chemical kinetic viewpoint[4]. Under natural conditions, however, plants are rarely exposed to a single pollutant. In the severely polluted roadside environments of northern countries several gaseous pollutants deriving from automobile exhausts influence the plants, together with sodium chloride used as de-icing salt. Excessive soil salt may affect the health of plants by inducing osmotic stress, via specific ion toxicity, through changes of pH, by creating a nutrient imbalance or by changing soil permeability or structure[5]. The relevance of the combined effect of salt and gaseous pollutants prompted us to demonstrate the influence of different salinity levels in the root medium on the uptake of NO_2 by leaves of *Phaseolus vulgaris* L. Seeds of *Phaseolus*