

To detect viral inhibitory activity in the coralloid root extract three methods were used. (1) Extract was with different virus inocula in equal amounts and kept for 15 min. Then the mixture was inoculated on 10 leaves of *Chenopodium amaranticolor* plants (the control consisted of each virus mixed with equal volume of distilled water instead of the extract). (2) Extract was applied on 10 leaves of *C. amaranticolor* 24 h before virus inoculation. (3) Extract was applied on 10 leaves of *C. amaranticolor* 24 h after virus inoculation. Leaves of control plants were similarly rubbed with distilled water instead of the extract. Lesions were counted 7 days after virus inoculation in the case of PVX, TMV and TRSV; and on the 10th day after virus inoculation in the case of PVY and TAV.

All the experiments were performed in an insect-free glasshouse at about $24 \pm 6^\circ\text{C}$ (63% humidity, 16-h-day and 18,000 lx). The data were analyzed statistically by the test of comparison between the control and the individual treatment to test for the significance of the activity of the extract⁶. Percent inhibition was calculated by the formula $(C-T)/C \times 100$, where C is the number of lesions on control leaves and T is the number of lesions on treated leaves. The result of virus inhibition of tomato plants by the coralloid root extract are summarized in the table.

The results in the table indicate that extracts of coralloid root of *Cycas revoluta* showed significant inhibitory activity (19–100%) at different dilutions of extract when applied 24 h be-

fore virus challenge or when mixed with virus inoculum before virus challenge; no such inhibition was observed when the extract was applied 24 h after virus challenge in either hypersensitive or systemic hosts. The extract did not show any inhibitory activity in a systemic host (tomato) against TAV. The root extract was completely inactivated at 1:1000 dilution.

The average number of local lesions varies with the viruses viz. 82 ± 11.6 , 68 ± 9.3 , 83 ± 5.2 , 96 ± 6.8 , 76 ± 9.1 produced on *C. amaranticolor* by PVX, PVY, TMV, TAV and TRSV respectively in control sets of plants (without inhibitor). The 100% protected plants were found to be disease free.

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Effects of TA [4-ethoxy-1-(p-tolyl)-s-triazine-2,6-(1H,3H)-dione] on growth, antheridium differentiation and gibberellin uptake of gametophytes of *Anemia phyllitidis* L. Sw.

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Summary. Synergistic effects of TA on gibberellin-dependent reactions in spermatophytes are not detectable in gametophytes of the fern *Anemia phyllitidis* where gibberellin substitutes for antheridiogens with regard to induction of male sexual organs and dark germination. In this archegoniate TA caused a significant reduction in growth rate and morphogenesis of the gametophytes, inhibitions that were not abolished by simultaneous application of gibberellin.

Key words. Fern; *Anemia phyllitidis*; antheridium differentiation; TA (4-ethoxy-1-(p-tolyl)-s-triazine-2,6-(1H,3H)-dione); gibberellin uptake.

Some derivatives of isourea and triazinone respectively show synergistic activity with gibberellins in the rice seedlings test^{1–3}. Since there is increasing evidence for a close relationship between the sexual pheromone system of schizaeaceous ferns and the gibberellin reactions in higher plants^{4,5} it seemed of interest to investigate the mutual effects of gibberellins and triazinones in the *Anemia* bio-assays (method: ref. 6).

In the *Anemia* system TA shows no synergistic reaction with gibberellin A₃ (GA₃) either on antheridium differentiation or dark induction of spore germination (tables 1, 2). Instead of

stimulating these processes, as it does in rice seedlings, the triazinone derivative cause a significant retardation of cell division in spores of *A. phyllitidis* when applied to the medium in concentrations ranging from 10^{-5} to 10^{-4} M (table 1). This inhibition was not reversed by simultaneous application of GA₃. In addition to a reduction of the rate cell division, TA showed morphogenetic effects in delaying the induction of two-dimensional growth leading to elongated gametophytes (fig. a + b). If one presupposes an identity of the primary reactions in the fern and spermatophyte system, the synergistic effect of TA

Table 1

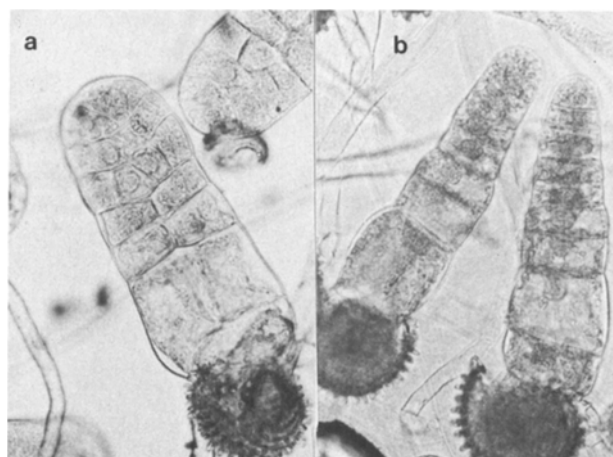
TA (M)	day 12 cell number	d ₅₀	CCN
0	16.4 ± 0.2	8	12.2 ± 0.1
10 ⁻⁵	14.0 ± 0.2	8	12.1 ± 0.2
5 × 10 ⁻⁵	10.8 ± 0.1	9	11.2 ± 0.1
10 ⁻⁴	8.2 ± 0.1	11	11.5 ± 0.2

Effects of TA on cell division and of TA and GA₃ on antheridium induction and critical cell numbers (CCN) in *A. phyllitidis* (continuous white light; $22 \pm 0.1^\circ\text{C}$). d₅₀, days until 50% of the prothallia differentiate antheridia.

Table 2

TA (M)	% germination
0	32.8
10 ⁻⁶	34.5
10 ⁻⁵	27.6
10 ⁻⁴	23.1

Effects of TA and GA₃ (10⁻⁶ g/ml) on dark germination of spores of *A. phyllitidis*. % germination after 10 days of culture ($21 \pm 0.1^\circ\text{C}$).



Growth inhibition and morphogenetic influence of TA on gametophytes of *A. phyllitidis*. a) control, b) retardation of cell division and induction of two-dimensional prothallium form by 10^{-4} M TA, 12-day cultures in continuous white light; 22 ± 0.1 °C.

and gibberellins in the rice assay should be based on an interaction of the reactions leading to shoot growth rather than on a synergistic influence at the hormone binding site. Indeed, Takeno et al.³ explained the synergistic effects of TA in the dwarf rice test by a combined action of this substance on the uptake and the metabolism of GA_4 . The determination of these two processes is very difficult in the *Anemia* system because, compared to tissues of higher plants, the rate of gibberlin uptake into cells of the fern gametophyte is extremely low. After 7 days of culture on a medium containing a hormone concentration of 1.5×10^{-5} M TA as compared with untreated controls on the basis of total uptake per prothallium

Table 3

	dpm/g fresh wt
control	11060 11020
10^{-4} M TA	10350 10100

Effects of 10^{-4} M TA on the uptake of GA_3 (1.5×10^{-5} M + 0.1μ C C^{14} GA_3 ; spec. act. $1,7,12,18^{-14}$ C 5–15mCi/mmol). Application of hormone and hormone + TA respectively to groups of 15-day-old prothallia. Culture in continuous white light at 22 ± 0.1 °C. Extraction after 7 days of feeding.

or g fresh weight (table 3) although this TA concentration already inhibits cell division of the prothallia.

Despite a far reaching correspondence of the reaction-system between fern antheridiogen and many gibberellin-mediated reactions of spermatophytes, there are distinct differences with respect to TA-treatment in the reactivity of the mechanisms of the pheromone and hormone transport.

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Partial purification and some characteristics of hamster molar alkaline phosphatase

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Summary. A rapid 3-step method is given to purify partially hamster molar alkaline phosphatase. Molecular weight was 50,200 and isoelectric point 3.7. The alkaline phosphatases in the mesenchymal and ectodermal parts of the tooth are probably identical. **Key words.** Hamster, Syrian; *Mesocricetus auratus*; alkaline phosphatase, molar.

Alkaline phosphatase (AP) has been related to the process of mineralization in bone² and teeth³. Isolation and characterization of AP from various tissues revealed the heterogeneity of the enzyme⁴ with respect to molecular weight, isoelectric point, substrate specificity and stability. Little is known, however, about the characteristics of AP from dental tissue^{5–7}. Moreover, the purification methods described are laborious. For future immunohistochemical localization of AP in hamster molar tooth germs, it will be necessary to obtain relatively pure enzyme. Therefore, we developed a simple and rapid procedure to purify hamster molar AP up to 121 times.

Materials and methods. First maxillary and mandibular molars were dissected from 3–4-day-old Syrian hamsters (*Mesocricetus auratus*) and collected on solid CO_2 . Between 100 and 150 molars were used for each experiment. They were homogenized in 10 ml bidistilled water at 0–4°C, using a Potter tube with glass pestle. After homogenization 4 ml n-

butanol was added⁴ and the mixture stirred for 2 h at 0–4°C. The phases were separated by centrifugation (15 min, $3000 \times g$, 0–4°C) and the water phase collected. This water phase was centrifuged at high speed (30 min, $190,000 \times g$, 0–4°C) and the resulting supernatant reduced in volume by lyophilization (this procedure also removes the remaining butanol). This supernatant was used for assessing some properties of AP and for further purification. To determine the molecular weight of the AP, the high-speed supernatant was further purified by means of high pressure gel permeation chromatography. The G 2000 SW column was calibrated with molecular weight markers in the range 94 kD to 255 D. To determine the isoelectric point of the AP a high-speed supernatant was chromatofocussed at 0–4°C. Since hamster molar AP activity decreased with time at low pH, the activities found in the eluate were corrected for the time they had been at low pH. In the various fractions AP activity was determined using p-nitrophe-