ucts. The results indicate that during anaerobic decomposition, DDT was converted directly to DDD, and further breakdown did not result in an appreciable buildup of any of the other identified products.

The total amount of radioactivity in the partitioned *n*-hexane layer of the soil extract decreased with increasing length of incubation. After 2 and 4 weeks of incubation, 29 and 43 percent, respectively, was unaccounted for as products soluble in *n*-hexane. Part of the activity unaccounted for in the *n*-hexane was found in the water layer after partitioning the other products into the *n*-hexane. Isolation and identification of water-soluble compounds have not been made. No activity was found in the hexane trap or as $C^{14}O_2$ (NaOH trap). Therefore, DDT was dechlorinated to DDD, and further degradation products included some water-soluble materials.

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2. Abbreviations used are: DDT: 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; DDE: 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene; DDD: 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane; DBP: 4,4′-dichlorobenzophenone; DDA: bis-(*p*-chlorophenyl)acetic acid; BA: *p*-chlorobenzoic acid; kelthane: 1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethanol; and DBM: 4,4′-dichlorodiphenylmethane.
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9. Pawnee silt loam with the following properties: *p*H, 5.2; total nitrogen, 0.154 percent; organic matter, 3.03 percent; and surface area, 142 m^2/g.
10. NCS, a strong organic base (quaternary ammonium salt dissolved in toluene) developed by Nuclear of Chicago, was used to absorb CO_2 for counting in liquid scintillation. Trade names are included as a matter of convenience to the reader, and such inclusion does not constitute any preferential endorsement of products named over similar products available on the market.
11. Counting solvent: 4 g of PPO (2,5-diphenyloxazole) and 50 mg of POPOP [*p*-bis(2-5-phenyloxazolyl)benzene] in 1 liter of toluene.
12. Gas chromatography: A 1.8-m pyrex glass column (outside diameter, 6 mm; inside diameter, 4 mm) was packed with equal portions of 10 percent Dow-Corning 200 silicone fluid [(12,500 centistokes) on 80/90 mesh] on Anakrom ABS and 15 percent QF-1 on Gas Chrom Q. Operating conditions of the chromatograph were: column temperature, 210°C; detector, 235°C; carrier gas (He) flow rate, 100 ml/min.
13. Research was conducted in cooperation with Colorado Agr. Exp. Sta. This report is scientific journal series No. 1174.

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Anemia phyllitidis: Inducibility of Physiological State Antagonistic to Antheridium Formation

Abstract. *It has been reported that a preparation obtained from prothalli of the fern species* Anemia phyllitidis *controls antheridium formation in this species. If this same preparation is applied at the spore stage it induces a physiological state antagonistic to antheridium formation. The inhibitory state is inducible only at a very early stage of development, but, once induced, it remains manifest at much later stages. Quite possibly, the inhibitory state arises in response to the factor that induces formation of antheridia. The inhibitory state may have an important function in fern development.*

Prothalli of *Pteridium aquilinum* elaborate a hormone that controls antheridium formation in many if not all species of the fern family Polypodiaceae (*1*, *2*). This hormone has been isolated and termed an antheridiogen (*3*). Native antheridiogens have also been demonstrated in the fern species *Blechnum gibbum*, *Lygodium japonicum*, *Anemia phyllitidis* (*4*), and *Anemia hirsuta*.

The antheridiogen of *P. aquilinum* does not affect *A. phyllitidis*, a species belonging to the fern family Schizaeaceae. The prothalli of this species elaborate a different antheridiogen (A_{An}, *4*). The phytohormone gibberellic acid (A_3) also induces formation of antheridia in *A. phyllitidis* (*5*, *6*). However, like A_{An} (*4*), A_3 has no effect on a species used to assay for the antheridiogen of *P. aquilinum* (*2*). Other gibberellins also cause antheridium formation in *A. phyllitidis*, some of them at an exceedingly low concentration (*7*, *8*). The antheridiogen A_{An} does not seem to be identical with any of the several tested gibberellins (*9*).

The sensitivity of fern prothalli to antheridiogen varies with the developmental stage (*1*–*6*). If *Anemia* prothalli are exposed to A_{An} at the 0-day stage (before, or immediately after, spore inoculation), then the interval between the appearance of antheridia at the highest concentration of the antheridiogen and that at the lowest concentration is 14 days. In contrast, the corresponding interval is approximately 1 day if A_{An} is supplied at the 11-day stage (*10*). If we take this interval as a basis for comparison, the sensitivity of 0-day-old prothalli to differences in antheridiogen concentration exceeds that of 11-day-old prothalli by a factor of about 14. The delayed appearance of antheridia in prothalli exposed at the 0-day stage might be interpreted to mean that the juvenile prothalli are refractory to the action of A_{An}. If this were true, prothalli exposed at the 0-day stage would be expected to form antheridia no later than the time at which they are formed by prothalli exposed at the 11-day stage. Instead, prothalli exposed to the lower concentrations of A_{An} at the 0-day stage formed antheridia much later than prothalli exposed at the 11-day stage did, and they also formed a much larger number of vegetative cells before they attained the antheridial phase (*10*) (Table 2). Clearly, an inhibitory state becomes operative in the juvenile prothallus, greatly delaying the appearance of antheridia but permitting the continuance of vegetative cell division. This inhibitory state may not be an obligatory characteristic of development but may be, instead, induced by the preparation containing A_{An}. On this assumption, inducibility of the inhibitory state decays before the prothallus attains the 11-day stage, but, once it is induced in the juvenile prothallus, the inhibitory state remains manifest beyond the stage at which it ceases to be inducible.

For all experiments, prothalli were cultured in 50-ml erlenmeyer flasks on 10 ml of an agar-solidified, inorganic medium. Conditions of culture and the method of spore sterilization have been described (*11*). The antheridiogen A_{An} was applied as the liquid medium harvested from 7-week-old cultures of *A. phyllitidis*. It induced formation of antheridia to a dilution of one part in a thousand in most spore samples; it is termed the active preparation below.

Prothalli were first grown for 11 days, some on nonsupplemented medium, others on medium supplemented with dilutions of the active preparation at full strength ranging from 1:100 to 1:3000. Eleven days after spore inoculation, prothalli grown on the nonsupplemented medium were transferred to the 11-day-old cultures of prothalli grown on supplemented medium. Prothalli on agar slivers about 60 mm^2 and 2 mm thick were transferred from the nonsupplemented cul-

Table 1. Differential behavior of prothalli exposed to A_{An} at 0-day and 11-day stages upon transfer to same medium. Recorded numbers are averages of numbers of individuals bearing antheridia in each of four samples of 30 observed prothalli. A, grown first on supplemented medium; B, grown first on nonsupplemented medium.

Dilution, *Anemia* medium	Days after transfer				
	A			B	
	2	6	12	2	12
	Experiment 1				
1:100	0.25	2.75	26.00	25.25	30.00
1:300	.00	0.00	16.00	25.25	28.00
1:1000	.00	.00	1.25	9.25	14.00
1:3000	.00	.00	0.00	0.00	0.00
	Experiment 2				
1:100	0.00	1.75	16.75	19.75	29.50
1:300	.00	0.00	9.25	16.50	22.00
1:1000	.00	.00	2.00	3.25	8.50
1:3000	.00	.00	0.00	0.00	0.00

tures (two slivers per flask) to areas of the agar surface free of prothalli in the supplemented cultures (Table 1, experiment 1).

In another experiment, prothalli at the 11-day stage were transferred to culture flasks with freshly prepared medium supplemented with various dilutions of the active preparation. Each flask received two agar slivers with prothalli first grown for 11 days on medium supplemented with the active preparation at the same dilution and two agar slivers with prothalli grown for 11 days on nonsupplemented medium (Table 1, experiment 2).

Prothalli exposed to A_{An} at the 0-day stage form antheridia later than do prothalli exposed at the 11-day stage, even if they are cultured on the same medium (Table 1). Clearly, the results are consistent with the hypothesis that exposure at the 0-day stage causes the induction of a physiological state inhibitory to antheridium formation. However, the possibility that an inhibitory state of very brief duration becomes operative even in the unexposed prothallus must be considered. If this were true, exposure of the 0-day-old prothallus to the preparation containing A_{An} would be effective by greatly lengthening the duration of the inhibitory state.

I studied at what stage of development the inhibitory state ceases to be inducible. *Anemia* prothalli were exposed to A_{An} at the 0-day (immediately after spore inoculation), 4-day, 6-day, and 11-day stages. Three-tenths milliliter of active preparation was added at strengths calculated to give dilutions in the medium of 1:30, 1:100, 1:300, 1:1000, and 1:3000. In prothalli exposed at the 0-day stage, the interval between the appearance of antheridia at the highest concentration of A_{An} and the appearance of antheridia at the lowest concentration is more than 14 days (Table 2). In contrast, in prothalli exposed at the 4-day stage, the corresponding interval is about 1 day. Clearly, the inducibility of the inhibitory state has already largely disappeared in most of the 4-day-old prothalli. However, with development from the 4-day stage to the 11-day stage, the interval between exposure to A_{An} and the emergence of antheridium initials is shortened by a roughly equal amount at all concentrations of A_{An} from about 3 days to less than 2 days (Table 2). It cannot yet be decided whether the observed decay of the high sensitivity to differences in concentrations of A_{An} between the 0-day stage and the 4-day stage has the same physiological basis as the subsequent, largely concentration-insensitive reduction of the lag period.

The terminal (apical) cells of juvenile individuals give rise to an antheridium only in response to the highest concentration of antheridiogen if the prothalli are exposed to A_3 or A_{An} at the 0-day stage (*5*, *11*). At lower concentrations, the sex organs arise from more basal cells. In contrast, many of the prothalli exposed at the 4-day stage form terminal antheridia even at the lowest effective concentration of A_{An}, that is, at a concentration 10 to 30 times lower than that causing formation of terminal antheridia by prothalli that are exposed at the 0-day stage. Clearly, the inhibitory state induced in the juvenile prothallus has a strong effect on the apical cell.

Table 2. Relation of A_{An} concentration to onset of antheridium formation in prothalli exposed to A_{An} at the 0-day, 4-day, 6-day, and the 11-day stage. Recorded numbers are averages of numbers of individuals bearing antheridia in each of four samples of 30 observed prothalli.

Dilution, *Anemia* medium	Days after exposure at stage											
	0-day						4-day		6-day		11-day	
	4	8	12	16	20	22	2	3	1	2	1	2
1:30	0.0	8.75					0.0	13.25	0.0	7.50	1.25	25.25
1:100	.0	0.25	0.25	2.75			.5	11.75	.0	7.75	0.75	24.75
1:300	.0	.00	.00	0.00	4.50	9.75	.0	7.50	.0	7.25	.00	23.50
1:1000	.0	.00	.00	.00	0.00	2.50	.0	1.75	.0	2.25	.25	16.25
1:3000	.0	.00	.00	.00	0.00	0.00	.0	0.00	.0	0.00	.00	0.50
Control	.0	.00	.00	.00	.00	.00	.0	.00	.0	.00	.00	.00

Table 3. Attempt to separate a factor inducing the physiological state antagonistic to antheridium formation from A_{An}. Solvent mixture 1: isopropanol, water, and ammonia, 8 : 1 : 1; solvent mixture 2: water and isopropanol, 8:2. Conditions of assay: (1) exposed to A_{An} at 9-day stage; observed for antheridia at 12-day stage. (2) Exposed at 0-day stage; observed at 12-day stage. (3) Exposed at 0-day stage; observed at 20-day stage. Recorded numbers are averages of individuals bearing antheridia in each of two samples of 30 prothalli.

Section of chromatogram	Condition of assay		
	1	2	3
	Solvent mixture 1		
1–7, 9–10	0.0	0.0	0.0
8	18.5	.0	6.5
	Solvent mixture 2		
1–17, 20	0.0	0.0	0.0
18	22.5	.0	8.5
19	2.0	.0	0.0

Chromatography was used to study the nature of the substance that induces the inhibitory state. Descending chromatography on Whatman filter paper No. 1 with solvent mixtures 1 (isopropanol, water and ammonia, 80:10:10) and 2 (water and isopropanol, 4:1) (*3*) was chosen. Fifty microliters of an ethanol extract with an activity ten times that of the active preparation at full strength were spotted. Chromatography was stopped when the solvent front had reached a distance of 24 or 48 cm from the origin. The chromatograms were cut into 10 or 20 sections, respectively, each section being eluted with 6 ml of water. Eluate (5.1 ml) from each section of the chromatogram was used for assay, 1.7 ml being added to each of three culture flasks. Two sets of assay flasks were inoculated with spores of *A. phyllitidis*, one set being observed at 12 days for antheridium formation, the other set being observed at 20 days for antheridium formation. To the third set of assay flasks were added two agar slivers with 9-day-old prothalli grown on nonsupplemented medium. These prothalli were observed for antheridium formation 3 days after transfer (12 days after inoculation).

The relative flow rates for the antheridium-inducing activity (Table 3) corresponded to those reported earlier for the two solvent mixtures used (*11*). Antheridia arose on prothalli exposed to A_{An} at the 9-day stage and assayed at the 12-day stage, and on prothalli

exposed at 0-day stage and assayed at the 20-day stage. In contrast, with prothalli exposed at the 0-day stage and assayed at 12-day stage none of the eluates caused antheridium formation within the specified period of time. The results are interpreted to mean that the sections of the chromatogram which caused antheridium induction also caused the induction of the physiological state inhibitory to antheridium formation. Clearly, a substance inducing the inhibitory state was not separated from A_{An}. The two solvent mixtures used led to the chromatographic separation of the three native fern antheridiogens thus far demonstrated (*12*). The possibility cannot be excluded that the inhibitory state is induced by A_{An}.

Such a hypothesis seems paradoxical. The described phenomenon might be functional in preventing the precocious attainment of the sexual phase. However, the inducibility of the inhibitory state has already largely decayed at the 4-day stage. The prothalli developed from the germinated spores then comprised an average of 1.5 green vegetative cells (only cells seen outside the spore coat were counted). The first spores germinated 3 days after inoculation. Quite likely, the peak in the inducibility of the inhibitory state begins to decline even before the spore germinates. It may be stressed that A_3 (*5*) and medium harvested from *Anemia* cultures (*11*) not only cause antheridium formation but also can cancel the light requirement for spore germination. In all likelihood, the native factor that induces germination in the dark is identical with A_{An} (*11*). In nature, recently shed spores are probably exposed to A_{An} secreted by prothalli developed from spores shed earlier. Accordingly, I propose that the inducibility of a state antagonistic to antheridium formation may be functional in the prevention of "hybrid metabolism," that is, the simultaneous occurrence of physiological states leading to germination and to formation of antheridia. A consideration of the above situation is valid regardless of whether or not light promotes germination by causing the synthesis of A_{An}. At a genetic level such a phenomenon would have its parallel in the finding that activation at one gene site may lead to suppression at another.

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Gaseous Hydrocarbons in Sea Water: Determination

Abstract. *Low-molecular-weight hydrocarbons in the C_1 to C_4 range have been detected and measured in sea water. Methane shows by far the highest concentration, followed in order by the C_2, C_3, and C_4 fractions. In general, the concentration tends to decrease with depth, but there is a strong indication that a maximum may exist about 30 to 50 meters below the surface.*

We report what we believe to be the first determinations of C_1 to C_4 low-molecular-weight hydrocarbons in waters of the open ocean. The presence of methane is well established in freshwater lakes and marshes (*1*). The presence of methane was recently reported in Lake Nitinat (*2*), an anoxic fjord on Vancouver Island; it apparently occurred only in the oxygen-depleted zone where H_2S also was found. Similar findings have been reported in the anoxic areas of the Black Sea. However, nothing has been reported of low-molecular-weight hydrocarbons other than methane.

With the development of a new and and extremely sensitive gas-chromatographic technique (*3*), we have succeeded in identifying and measuring methane, ethane, ethylene, propane, propylene, *n*-butane, *iso*-butane, and some of the butenes in waters of the open ocean. Some of the chromatograms showed small peaks having longer retention times than the butenes had, indicating the presence of C_5 hydrocarbons. Our primary purpose was to evaluate the analytic method as applied to sea water, so that very few supporting oceanographic data were taken at the time of sample collection.

Samples were collected from two areas: the open Atlantic some 500 km west of Ireland, and the Gulf of Mexico just off the continental shelf, directly south of Mobile, Alabama. All sampling was with standard Nansen casts; upon retrieval, the sea water was immediately transferred to an all-glass 1-liter bottle by gravity flow, the bottle being filled to overflowing by means of a 6-mm copper tube extending to its bottom. A tapered ground-glass stopper was inserted in such a way so as to avoid entrapment of room air.

Analysis was by a technique in which the dissolved hydrocarbons are first stripped from solution by purging with helium, and then concentrated in cold traps containing appropriate adsorbents; they are subsequently released by increase in temperature and swept into the chromatograph by a second stream of helium carrier gas. With this technique, sample size is not restricted and very dilute solutions may be analyzed.

Two cold traps (−77°C) in series were used. In the first, activated alumina was used to trap all hydrocarbons except methane; in the second, activated charcoal was used to trap methane. When the stripping was complete, the traps were isolated by closure of appropriate valves, and their temperature was raised to approximately 90°C. Helium carrier gas was then used to strip each adsorbent, in turn, of the

Table 1. Concentrations ($\times 10^{-7}$ ml/liter) of hydrocarbons found in waters from the Gulf of Mexico (G) and the North Atlantic (A). Ethane and ethylene were not separable with the column used.

Depth (m)	Methane		Ethane and ethylene		Propane		Propylene		*iso*-Butane	
	G	A	G	A	G	A	G	A	G	A
0	676	474	45.0	99	2.4	1.9	9.3	5.9	5.9	2.9
30	2830	600	102	51.3	6.6	3.4	6.3	8.5	6.9	2.4
500	246	488	5.4	12.3	1.7	0.8	1.6	3.1	5.9	1.4