subsequently, fatty acids are transferred to phospholipid, primarily within mitochondria, prior to oxidation by way of the citric acid cycle. The significance of the changes in free fatty acid, cholesterol esters, and cholesterol is not clear.

These results provide the first direct evidence for oxidation of endogenous lipid by the perfused rat heart.

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Gibberellins: Their Effect on Antheridium Formation in Fern Gametophytes

Abstract. *Gametophytes of the fern* Anemia phyllitidis *respond to seven different gibberellins (A_1, A_3, A_4, A_5, A_7, A_8, and A_9) by forming antheridia. Gibberellin A_7 is active at concentrations as low as 5×10^{-10} g/ml. Species from two of the three other genera in the family Schizaeaceae respond similarly to gibberellin A_3. In contrast, nearly 40 species from 7 other families of ferns do not develop antheridia when supplied with gibberellin.*

In a series of three papers, Döpp (*1*) demonstrated that the bracken fern, *Pteridium aquilinum* (L.) Kuhn, secreted a biologically active substance which caused newly germinated gametophytes of bracken fern to form male reproductive organs, or antheridia. These distinctive reproductive structures are formed from single cells of the gametophyte by a series of unequal cell divisions (*2*). As shown in Fig. 1, each division is followed by striking differentiaton of the two daughter cells. The natural substance, which could initiate this series of divisions, was obtained by Döpp in aqueous extracts of the gametophytes or in the medium upon which gametophytes had been cultured for several weeks.

Although this substance was organ-specific, it was not species-specific, in that its presence in culture media led to the formation of antheridia in *Dryopteris filix-mas* (L.) Schott as well as in the bracken fern. Furthermore, aqueous extracts of *Dryopteris* elicited antheridia in *Pteridium*. In each instance, the ferns produced antheridia under conditions in which no antheridia were formed in extract-free control cultures. The response of more than 50 species of ferns from 8 major families was summarized by Voeller (*3*). Two additional antheridium-inducing substances, or antheridogens have been found in other families of ferns (*4*).

Chemical analysis (*5*, *6*) of pure, isolated antheridogen A, as the hormone is named, showed that the substance is of low molecular weight, and contains a carboxyl group and one unsaturated carbon-carbon bond. The pK_a of the acid is about 5.0. The properties of antheridogen A suggest that, among the chief groups of naturally occurring plant growth substances, it most resembles the gibberellins. Moreover, gibberellin A_3 has quite striking androecium- (male flower) promoting properties, when applied to various flowering plants (*7*). The possibility that the antheridogens are gibberellin-like substances is also interesting in that Kato *et al.* (*8*) reported the detection of a gibberellin-like substance in ferns, although in sporophytes rather than gametophytes.

The results of tests of the activity of gibberellin A_3 upon *Pteridium aquilinum* and *Onoclea sensibilis* L., the latter being particularly responsive to the antheridogen, have been consistently negative (*3*). The substance was tested over a wide range of concentrations, both in the presence and absence of such other growth substances as indole-3-acetic acid and 6-furfurylaminopurine (kinetin). Six other gibberellins, A_1, A_4, A_5, A_7, A_8, and A_9, showed no effect upon antheridium induction in *Onoclea* (*3*). A slight effect of gibberellin A_3 upon both antheridium and archegonium induction in *Aspidium oreopteris* was reported by Witsch and Rintelen (*9*). Evidently, the effect was not organ-specific, nor did these workers detect enhancement of the formation of reproductive organs in *Polypodium vulgare* L. or in *Pteridium aquilinum* grown on gibberellin-containing media.

Table 1. The response of species of the Schizaeaceae to gibberellin A_3, at a concentration of 5×10^{-5} g/ml.

Species	Gibberellin	Controls
Schizaea pusilla Pursh (*19*)	+	+
Lygodium japonicum (Thunb.) Sw. (*20*)	+	−
Anemia hirsuta (L.) Sw. (*21*)	+	−
A. pastinacaria Moritz	+	−
A. oblongifolia (Cav.) Sw.	+	−
A. tomentosa var. *mexicana* (Presl) Mickel	+	−
A. tomentosa var. anthriscifolia (Schrad.) Mickel*	+	−
A. jaliscana Maxon	+	−
A. rotundifolia Schrader (*20*)	+	−
A. phyllitidis (L.) Sw. (*22*)	+	−
A. phyllitidis (*20*)	+	−
Mohria caffrorum (L.) Desv. (*23*)	+	−

*Collected in South America by Lichtenstein.

In contrast with the slight, or complete lack of response of the species of fern prothallia tested, *Anemia phyllitidis* (L.) Sw. responds strikingly to gibberellin. Schraudolf (*10*) showed that 100 percent of the gametophytes of this member of the family Schizaeaceae produced antheridia when grown with gibberellin A_3 at concentrations of 5×10^{-6} g/ml, or higher. Indeed, 70 to 80 percent of the gametophytes bore antheridia when grown on media with a gibberellin concentration of

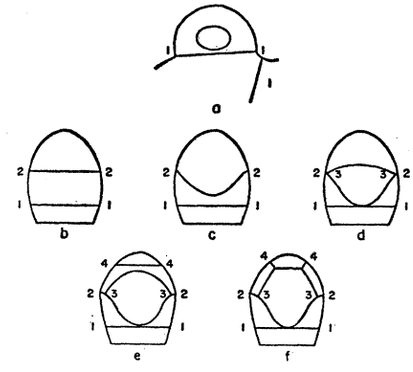

Fig. 1. Diagrammatic representation of the formation of an antheridium [after Davie (*2*)]. (*a*) The antheridial initial, showing a nucleus at its center. The initial is cut off from its parent cell by wall one (1). (*b*) The two-celled stage. (*c*) Depression of cell wall two (2). (*d*) Delimitation of the gametogenic, central region. (*e*) Formation of the "cap" cell and the donut-shaped "collar," or ring cell. (*f*) A mature antheridium.

Table 2. The response of *Anemia phyllitidis* and *Onoclea sensibilis* to seven gibberellins, each at a concentration of 5×10^{-6} g/ml.

Gibberellin	Response	
	Anemia	*Onoclea*
Control	−	−
A_1	+*	−
A_3	+	−
A_4	+	−
A_5	+*	−
A_7	+	−
A_8	+†	−
A_9	+	−

* Response delayed, but unequivocal. † Antheridia formed only at higher concentrations.

5×10^{-7} g/ml. In all instances, the controls were free of antheridia. Schraudolf obtained similar results with *A. rotundifolia* and *Lygodium japonicum* (Thunb.) Sw. These observations have been confirmed and extended by Voeller (*3*).

In view of the various reactions of different species of ferns to gibberellins, it seemed of interest to study the responses of ferns from a much wider taxonomic range, particularly those belonging to the family Schizaeaceae, and to determine the effect upon *Anemia phyllitidis* of the several other gibberellins.

Small samples of spores, under sterile conditions, were inoculated into 50-ml Erlenmeyer flasks containing 10 ml of Moore's (*11*) medium supplemented with Gorham's (*12*) trace elements and solidified with 0.9 percent agar. Cultures were grown at 22°C under continuous illumination at an intensity of about 22 lumen/m^2. Aqueous gibberellin solutions were sterilized by filtering. Three duplicates were made of each of the experimental flasks and two of each of the controls.

At the concentration of gibberellin A_3 tested, 5×10^{-5} g/ml, which is 500 times greater than that required to induce antheridia in *Anemia phyllitidis*, there was no stimulatory effect on the formation of antheridia in the following seven families of ferns.

Osmundaceae:
Osmunda claytoniana L.
O. cinnamomea L.

Cyatheaceae:
Alsophila (Cyathea) australis R. Br.

Pteridaceae:
Cibotium barometz (L.) J. Sm. (*13*)
Dennstaedtia bipinnata (Cav.) Maxon (*13*)
Microlepia speluncae (L.) Moore
Pteridium aquilinum (L.) Kuhn (*9*)
Pteris cretica L.
P. tremula R. Br. (*14*)
P. longifolia L.
Pellaea viridis (Forsk.) Prantl
P. hastata
Hemionitis arifolia (Burm.) Moore (*13*)
Pityrogramma hybrida Domin. (*13*)
Adiantum pedatum L.

Davalliaceae:
Nephrolepis cordifolia (L.) Presl (*13*)
Davallia (Scyphularia) pentaphylla Blume

Aspidiaceae:
Onoclea sensibilis L.
Polystichum tsus-simense (Hook.) J. Sm. (*13*)
P. acrostichoides (Michx.) Schott
Dryopteris filix-mas (L.) Schott
D. dilatata Gray (*15*)
Tectaria macrodonta (Feé) C. Chr.
T. heracleifolia (Willd.) Underw. (*14*)
Athyrium filix-femina (L.) Roth
Thelypteris (Cyclosorus) dentata (Forsk.) E. St. John (*13*)
Cyrtomium (Phanerophlebia) falcatum Presl (*13*)

Blechnaceae:
Blechnum brasiliense Desv. (*13*)
B. occidentale L. (*15*)
Woodwardia (Anchistea) virginia (L.) Sm.
Doodia media R. Br.

Polypodiaceae:
Polypodium feei (Bory) Mett. (*13*)
P. vulgare L. (*9*)
P. (Phlebodium) aureum J. Sm. (*13*)
P. polycarpon Cav. (= *Microsorium punctatum* (L.) Feé)
P. (Goniophlebium) subauriculatum Blume
P. (Pessopteris) crassifolium L. (*10*)
Drynaria quercifolia (L.) J. Sm. (*13*)

Gametophytes were examined weekly until they had attained mature form—that is, until they had developed a well-defined apical meristematic notch (heart shape). Thus, it must be concluded that the effect of gibberellin A_3 in antheridium formation shows high taxonomic specificity and is of no clear significance in non-schizaeaceous ferns. It is of special interest that *Osmunda* and the cheilanthoid ferns *Pellea, Pityrogramma,* and *Adiantum* showed no response, since these two groups of ferns are thought to have phylogenetic affinities with the Schizaeaceae (see *16–18*).

The effects of gibberellin A_3 upon members of the family Schizaeaceae are summarized in Table 1 and Fig. 2. Every species of *Anemia* tested showed a striking response to the presence of gibberellin, but the time between germination and the appearance of antheridia varied from species to species. *Anemia phyllitidis* (from New York) and *A. rotundifolia* developed male reproductive organs within 3 or 4 days of germination, whereas more than 2 weeks were required for the development of antheridia in *A. pastinacaria.* None of the control gametophytes, grown in the absence of gibberellin, bore antheridia even after many weeks. Similarly, *Lygodium japonicum* and *Mohria caffrorum* developed antheridia under the influence of gibberellin. However, in view of the close taxonomic relationship between *Anemia* and *Mohria* (*17*), it was interesting that *Mohria* developed antheridia only several weeks after germination of the spores. Thus, it was distinctly slower than any species of *Anemia.* At the concentration of gibberellin used, *Mohria* showed fewer antheridia per thallus than did *Anemia phyllitidis,* and these were often green in color. Con-

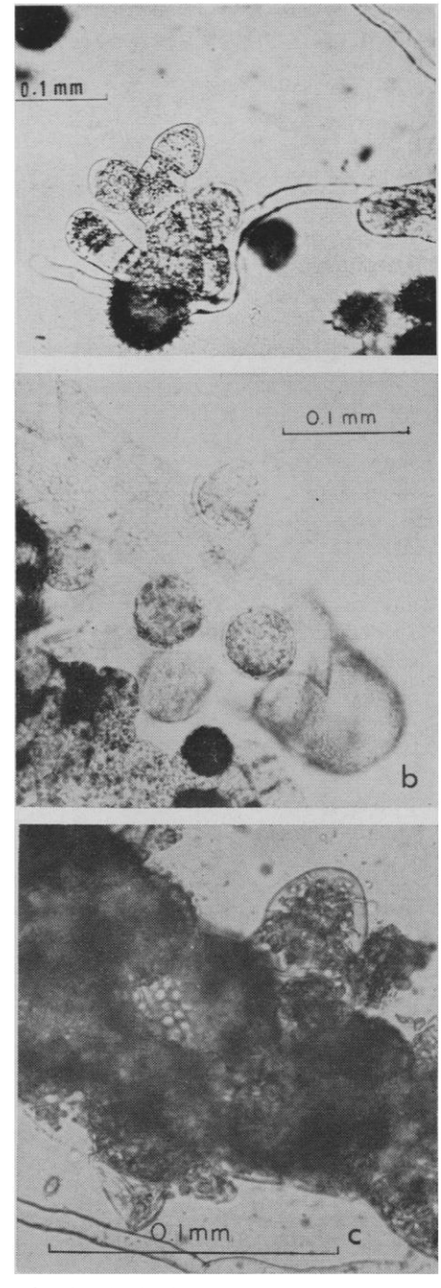

Fig. 2. Antheridia of ferns raised in the presence of gibberellin A_3 (5×10^{-5} g/ml). (*a*) *Anemia phyllitidis.* (*b*) *Lygodium japonicum.* Five antheridia are visible. (*c*) *Mohria caffrorum.*

trols of both *Lygodium* and *Mohria* grew well but none of them produced antheridia.

Spores of *Schizaea pusilla* germinated several weeks after inoculation and grew into long, rarely branched filaments or protonemata. This mode of growth is a characteristic of the genus (*24*). After many weeks of growth, scattered filaments developed antheridia. However, antheridia were also present in similar numbers in control flasks lacking gibberellin. Attempts are being made to obtain other species of the relatively primitive genus, of which *S. pusilla* is the only member which occurs north of the Tropic of Cancer (*17*).

Samples of several of the naturally occurring gibberellins (*25*) were tested for their influence upon the growth of *Anemia phyllitidis* and *Onoclea sensibilis*. The results are shown in Table 2. The minimum effective concentration to which the ferns responded varied greatly. Gibberellins A_4, A_7, and A_9 were particularly effective. The lowest concentration of gibberellin A_3 found to be effective was 10^{-7} g/ml, in agreement with the results of Schraudolf (*10*). Gibberellin A_7 was biologically active at concentrations as low as about 5×10^{-10} g/ml, a value similar to the lowest effective concentration (*5*) of pure antheridogen A.

Onoclea sensibilis did not respond to any of the gibberellins tested (Table 2). When the medium upon which this species had been grown was freed of gametophytes and reinoculated with *Anemia* spores, antheridia were formed on the *Anemia* gametophytes in every flask except the gibberellin-free controls. This eliminated the possibility that the gibberellins were inactivated in the medium to any appreciable extent by *Onoclea*.

Evidence has been presented by Voeller (*3*) that the seven gibberellins tested were not identical with the naturally occurring antheridogen A from *Pteridium* or antheridogen B from *Anemia phyllitidis*. The gibberellins and pure antheridogens were compared by thin-layer chromatography, the substances being detected by color tests and bioassay. The sensitivity of *Anemia phyllitidis* to the known gibberellins, and the rapidity and ease of growth of the fern suggest that it might be useful in qualitative assays of gibberellins in plant extracts.

The striking uniformity of the developmental stage of *Anemia phyllitidis*, when raised upon gibberellin-containing media, facilitates studies of the cytological and biochemical processes accompanying antheridium formation. The effects of various protein, nucleic acid and mitotic inhibitors upon these processes are being investigated. It is possible that the gibberellins or antheridogens are essential for several of the stages in antheridium development, rather than just in initiation of the organ. Such studies may help to elucidate the relationship between gene and hormonal action.

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Circadian Periodicity in the Concentration of Prolactin in the Rat Hypophysis

Abstract. *Prolactin activity in the hypophyses of rats maintained under standardized conditions was assayed in pigeons. The concentration of prolactin in the gland exhibited a circadian rhythm.*

A circadian periodicity in secretion of luteinizing hormone by the hypophysis of rats is indicated by the action of Nembutal, which blocks its release if administered at 2 P.M. but not when the same dose is given at 4 P.M. (*1*). Also, the concentration of corticotropin in the hypophysis of the mouse of strain C follows a circadian rhythm (*2*). In this study, a circadian periodicity in the concentration of prolactin in the hypophyses of rats was revealed by direct measurement.

Ninety-eight female Sprague-Dawley rats, each weighing 180 to 190 g, were kept for 1 to 2 weeks under standardized conditions: they were isolated in individual cages away from disturbances, exposed to light from 6 A.M. to 6 P.M., and kept at a constant temperature of 21°C. Groups of rats were killed at 2-hour intervals during the day and night, each group having been undisturbed for the preceding 48 hours. Within 1 to 2 minutes after removal from the standardized conditions, each animal was anesthetized with ether, the hypophysis excised, and the pars nervosa removed from it. The gland was frozen in liquid nitrogen and stored at −20°C. After thawing it was weighed, homogenized in 1.2 ml of distilled water, and divided into three 0.4-ml portions. Prolactin activity was measured by the method of Lyons as modified by Reece and Turner (*3*). One-tenth of a milliliter of homogenate was injected intradermally over the crop sac of pigeons, daily for 4 days, the homogenate from each rat being used for three pigeons. The pigeons were killed 5 days later and crop sac proliferation was estimated visually on a scale from 0 to 4 in increments of 0.25.