with *Methanobrevibacter smithii* ALI, and only weakly with *Methanobacterium bryantii* MoH and MoHG, and *Methanobacterium thermoautotrophicum* ΔH. Such a pattern indicates that these last-mentioned four strains share a determinant which is immunodominant in PS but not in ALI, MoH, MoHG, and ΔH. This idea is supported in the case of strains MoH and ΔH by their failure to elicit antibodies that cross-react with strain PS. Other similarities in the bacterial-wall composition and architecture (*6*) may also be involved in determining cross-reactions.

Multiple assay with antibody probes proved useful for identification and classification of two new isolates, designated *Methanobacterium thermoautotrophicum* GC1 and *Methanobrevibacter smithii* ALI. The strain GC1 is morphologically and physiologically similar to the type strain *Methanobacterium thermoautotrophicum* ΔH, and the immunological typing supports the inclusion of GC1 in the same species as ΔH. Although strain ALI is morphologically and physiologically similar to both *Methanobrevibacter ruminantium* strain M1 and *Methanobrevibacter smithii* PS, it is more closely related immunologically to *Methanobrevibacter smithii* PS.

Immunological typing revealed other interesting features in relation to the classification of methanogens by Balch *et al.* (*3*). Although their 16*S* rRNA oligonucleotide catalogs showed no difference between *Methanosarcina barkeri* strains MS and 227, the strains are immunologically different. In contrast, while *Methanobacterium formicicum* strain MF, *Methanobrevibacter ruminantium* M1, and *Methanobrevibacter arboriphilus* strain DH1 are closely related to one or another of the Methanobacteriaceae according to the 16*S* rRNA catalog, they do not cross-react with any member of that family. The cross-reactions between *Methanospirillum hungatei* strain JF1 antibody and the two *Methanogenium* strains is noteworthy because the latter are marine forms that require NaCl for growth and differ in morphology from JF1. Our immunological evidence supports the family relationships of the genera indicated by the 16*S* rRNA catalog. Some pairs of cross-reactive strains [such as *Methanobacterium bryantii* MoH and MoHG (Fig. 1)] display similar patterns of reactivity. In such cases, antigenic identity can be confirmed or ruled out by using our antibody probes at other levels of sensitivity or by standard serial dilution analysis and selective adsorptions of the antiserums.

Our data support the recent classification of methanogenic bacteria based on information produced by molecular biology techniques (*3*, *7*). Although those techniques are important for establishing phylogenetic relationships among taxa, immunological methods offer a simple and quick alternative for identification of new isolates and their assignment to a family and, in most cases, to a genus. Quantification of antigenic relatedness and elucidation of the molecular basis of the antigenicity of the methanogens' cell wall, by means of antibody probes, including monoclonal (*11*), should furnish valuable information for a critical analysis of the evolutionary significance of methanogens, including the "adaptive sense" (*8*) of their heterogeneity.

EVERLY CONWAY DE MACARIO
MEYER J. WOLIN
ALBERTO J. L. MACARIO
Division of Laboratories and Research, New York State Department of Health, Albany 12201

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Lower Eocene and Paleocene Gentianaceae: Floral and Palynological Evidence

Abstract. *Lower Eocene flowers with* Pistillipollenites macgregorii *pollen represent the earliest megafossil evidence of the Gentianaceae. The Paleocene occurrence of* P. macgregorii, *the fossil's modern floral structure, and suggested trends in the evolution of pollen in the Gentianaceae indicate a considerably earlier origin for the family. Floral morphology typical of bee-pollinated flowers provides the earliest, albeit indirect, fossil evidence of bees.*

Flowers allied with the Gentianaceae have been discovered in the Wilcox Formation (Lower Eocene) of northeastern Texas. The flowers have a 22-mm diameter, sympetalous, open, funnelform-salverform, seven-lobed corolla (Fig. 1a).

Pollen preserved within the anthers matches an unusual type of dispersed pollen: *Pistillipollenites macgregorii* (*1*, *2*). Pollen grains isolated from the flowers are 22 μm in diameter, triporate, and are distinguished by gemmate ornamentation (Fig. 1, b and c). Pores are round to lolongate and are flanked by two elongate processes (Fig. 1c), possibly resulting from the fusion of gemmae, or are surrounded by discrete gemmae (*2*). Exine is intectate (Fig. 1b), except under the larger gemmae where there are short columellae (Fig. 1d). In areas with no ornamentation, the exine is 0.3 μm in thickness, and the largest gemmae are up to 2.4 μm in height. Although several staining techniques have been used on the fossil pollen, it is impossible to distinguish between the endexine and the ektexine or to determine whether the endexine is preserved at all.

Pistillipollenites has not been previously assigned to an extant family because it is a unique grain with no exact modern analog (*1*, *2*). Pollen with similar gemmate ornamentation is now known in a few species of four extant families; namely, the Boraginaceae (in several species of the genus *Tournefortia*) (*3*), the Euphorbiaceae (in several species of the genus *Zimmermannia*) (*4*), the Papilionoideae (in one species of *Herpyza*) (*4*), and in the Gentianaceae (in four species of the genus *Macrocarpaea*) (*5*–*8*). Pollen of *Zimmermannia* is tricolporate (*4*); pollen of *Herpyza* has apertures independent of gemmae and a granular

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exine structure (*4*); that of *Tournefortia* has no columellae associated with large gemmate processes (*3*), has large processes supported by raised platforms of exine, and has pores covered by ornamented ektexine layers (*3*). Pollen of four species of *Macrocarpaea* is most similar to the fossil, but has more gemmae (*6–8*) (Fig. 1e). Apertures of modern *Macrocarpaea* are similar (*5*) to those of the fossil and either surrounded by separate gemmate processes [as in *M. pachystyla* (Fig. 1e)], or flanked by elongate processes [as in *M. corymbosa* (*6*)]; the exine is intectate except in the area of the large gemmae where there are short columellae (Fig. 1f).

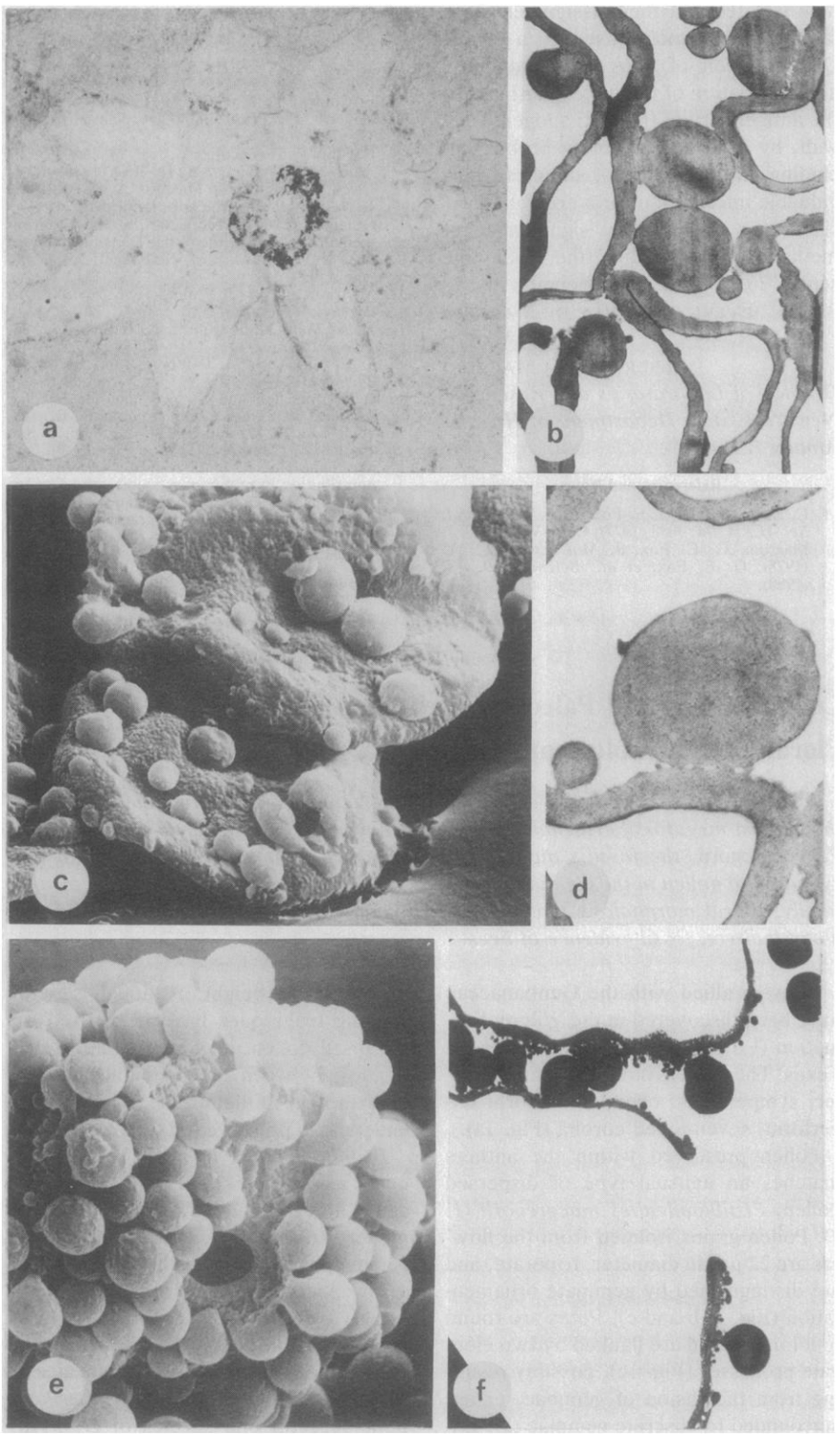

Fig. 1. (a) Overall view of fossil gentian showing a radially flattened somewhat distorted, seven-lobed sympetalous corolla (× 3.2). (b) TEM of several flattened *Pistillipollenites macgregorii* grains (× 5800). (c) Two pollen grains (*P. macgregorii*) from anthers of the flower illustrated in (a). Note the large gemmate processes and the pore (× 2690). (d) TEM of a gemma of *P. macgregorii* showing short columellae (× 13,250). (e) SEM of *Macrocarpaea pachystyla* pollen (× 3350). (f) TEM of *M. pachystyla* pollen (× 3500).

Modern flowers with sympetalous, funnelform-salverform, seven-parted corollas are found in only 11 families (*9*). The combination of floral morphology and pollen morphology of the fossil flowers is confined to four species of the Andean genus *Macrocarpaea* (Gentianaceae). None of the other species with gemmate pollen have similar corollas, and such floral morphology is not found in any of their families.

Pistillipollenites macgregorii is widely distributed in the Paleogene of the Northern Hemisphere and is known from the Eocene of British Columbia (*1, 10*) and the U.S.S.R. (*11*); the Lower Eocene of the Mississippi Embayment (*12*) and north central Europe (*13*); and the Paleocene of Texas (*14*), Alaska (*2*), and Europe (*13*). In view of the affinities of *Pistillipollenites*, the most intriguing report has been from the mid-Cretaceous (Cenomanian, Woodbine Formation) of Oklahoma (*15*). The original report included only a light micrograph of *Pistillipollenites*. Because of the ramifications of possible mid-Cretaceous gentians, we macerated (*16*) portions of Hedlund's type sections of the Woodbine Formation and then we isolated *Pistillipollenites* for scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The Cenomanian *Pistillipollenites* is tricolpate (*17*) and cannot be considered to represent the same taxon as Paleocene *Pistillipollenites*.

Paleocene *Pistillipollenites* represents the earliest occurrence of the Gentianaceae (*18*), and the modern aspect of the flowers from Texas implies an even earlier origin for the family. This is consistent with Nilsson's suggestion that gemmate pollen evolved in the Gentianaceae by reduction from reticulate ancestors (*6*).

Fossil floral morphology is suggestive of bee pollination within the Gentianaceae (*19*) and in angiosperm flowers in general (*20, 21*). Fossil bees are known only as early as the Lower Oligocene (*22*) and these flowers constitute the first evidence of bees. Finally, the affinities of an enigmatic pollen grain have been determined adding new biological significance to the dispersed pollen flora.

WILLIAM L. CREPET
Biological Sciences U-42, University of Connecticut, Storrs 06268
CHARLES P. DAGHLIAN
Department of Botany and Microbiology, University of Oklahoma, Norman 73019

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Growth Arrest and Morphological Change of Human Breast Cancer Cells by Dibutyryl Cyclic AMP and L-Arginine

Abstract. *The growth in vitro of human breast cancer cells, line MCF-7, was inhibited by a daily supplement of* L*-arginine (1 milligram per milliliter). Arginine acted synergistically with dibutyryl adenosine 3′,5′-monophosphate (cyclic AMP) (10^{-6} molar) to enhance the growth inhibitory effect: the cell replication ceased completely within 2 days after treatment. The growth arrest accompanied a change in cell morphology and was preceded by increases in the cellular concentration of cyclic AMP, adenylate cyclase, and type II cyclic AMP–dependent protein kinase activities as well as a decrease of estrogen binding activity. The results suggest that growth of human breast cancer cells is subject to cyclic AMP–mediated regulation and that arginine may play a specific role in this process.*

Human mammary carcinomas often regress after endocrine therapy. The regression has been linked to the presence of an estrogen receptor in the tumor (*1*) and, indeed, measurement of the receptor is now used extensively as an assay to identify patients likely to respond to endocrine therapy. However, about one-half of human mammary carcinomas containing estrogen receptor do not regress after ovariectomy (*2*). It is conceivable, therefore, that estrogen receptor may interact with other cellular regulators in the growth control of mammary tumors.

Studies from our laboratory suggested that regulation of the growth of hormone-dependent mammary tumors may depend on the antagonistic action between estrogen and adenosine 3′,5′-monophosphate (cyclic AMP). Cyclic AMP arrests whereas estrogen stimulates the growth of 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced rat mammary tumors (*3*). During growth arrest of the tumors after either hormone removal (ovariectomy) or treatment of the hosts with N^6,$O^{2'}$-dibutyryl adenosine 3′,5′-monophosphate (dibutyryl cyclic AMP), estrogen binding decreases whereas cyclic AMP binding and cyclic AMP–dependent protein kinase activity increase in the cytosol and nuclei of the tumors (*4*). Moreover, our recent studies (*5*) have shown that the growth inhibitory effect of dibutyryl cyclic AMP is enhanced by L-arginine which stimulates nicotinamide adenine dinucleotide (NAD)–dependent adenosine diphosphate ribosylation and adenylate cyclase activation in the hormone-dependent mammary tumors in vivo.

In the work reported here we explored (i) the role of cyclic AMP in the growth of human mammary cancer cells in vitro; (ii) the effect of arginine in the cyclic AMP system; and (iii) the synergistic action between arginine and dibutyryl cyclic AMP in the growth control.

We chose a human breast cancer cell line, MCF-7, as an experimental model. The MCF-7 cell line was derived from a hormone-dependent metastatic breast cancer (*6*). Although the growth of these cells does not apparently depend on exogenous estrogen, estrogen receptor is present (*7*) and antiestrogen (tamoxifen) strongly inhibits growth (*8*). Thus, MCF-7 cells appear to possess characteristics resembling those of hormone-dependent mammary tumors in vivo.

Daily supplements of L-arginine to MCF-7 cells in culture produced consistent growth inhibition. The growth inhibition was concentration dependent and was apparent within a few days. At a concentration of 1 mg of L-arginine hydrochloride per milliliter, the cell num-

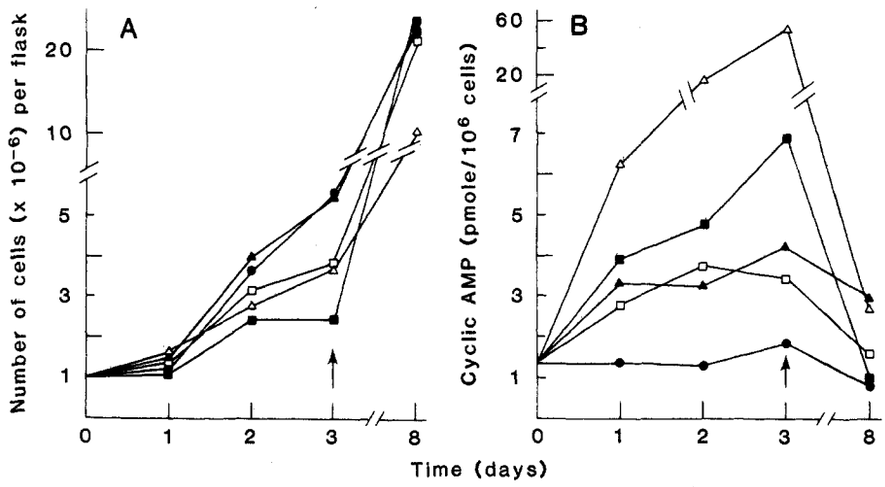

Fig. 1. Effect of L-arginine and dibutyryl cyclic AMP on the growth and cyclic AMP content of MCF-7 cells in culture. Symbols: ●, control; □, L-arginine hydrochloride (1 mg/ml); ▲, dibutyryl cyclic AMP (10^{-6} *M*); △, dibutyryl cyclic AMP (10^{-4} *M*); ■, dibutyryl cyclic AMP (10^{-6} *M*) plus L-arginine hydrochloride (1 mg/ml). The MCF-7 cells were obtained from Mason Research Institute (Rockville, Maryland) 1 day after seeding (1.0×10^6 cells per T_{75} Falcon flask). Cells were grown in the absence or presence of the additives in McCoy's 5A medium supplemented with bovine insulin (10 μg/ml), penicillin (100 U/ml), streptomycin (100 μg/ml), and fetal calf serum (10 percent); the medium was changed every 48 hours. The additives were provided daily starting at day 1 after seeding (0 time). The arrow indicates removal of the additives. The triplicate cell counts for each experimental point never varied by more than 15 percent. Cyclic AMP was measured by the radioimmunoassay according to the acetylation procedure (*22*) exactly as described (Collaborative Research Inc., Waltham, Massachusetts). Each point represents an average of closely agreeing duplicate determinations. The data represent one of several similar experiments that gave essentially the same results.

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