dose of 10 mg/kg. The intermediate position of teeth chattering in Wei's hierarchy, even though it occurred infrequently in our study, combined with the more frequent occurrence of abnormal postures and ear blanching, may be responsible for the lack of a significant reduction in Wei abstinence scores at a 5 mg/kg dose of THC.

These results suggest that further exploration of the therapeutic utility of the tetrahydrocannabinols in narcotic detoxification is warranted. The possibility that such compounds may offer advantages over the widely used methadone substitution technique (*8*) or the proposed use of neuroleptics such as haloperidol (*9*) deserves further study. Methadone, for example, produces physical dependence, and haloperidol produces extrapyramidal side effects as well as morphine-like abstinence signs in animals after repeated administration is terminated (*10*). On the other hand, physical dependence has not been associated with the use of cannabinoids in humans (*11*); toxic effects appear minimal (*12*) when these compounds are used for relatively short periods, as might be required during narcotic detoxification.

B. HINE
E. FRIEDMAN
M. TORRELIO
S. GERSHON

Neuropsychopharmacology Research Unit, Department of Psychiatry, New York University School of Medicine, New York 10016

References and Notes

1. W. R. Martin, *Pharmacol. Rev.* **19**, 463 (1967).
2. P. Lomax, *Proc. West. Pharmacol. Soc.* **14**, 10 (1971).
3. R. Adams, D. C. Pease, J. H. Clark, *J. Am. Chem. Soc.* **62**, 2194 (1940); R. Mechoulam, A. Shani, H. Edery, Y. Grunfeld, *Science* **169**, 611 (1970).
4. Formulated according to the method of R. D. Gibson and J. E. Tingstad [*J. Pharm. Sci.* **59**, 426 (1970)].
5. T. J. Cicero and E. R. Meyer, *J. Pharmacol. Exp. Ther.* **184**, 404 (1973).
6. E. Wei, *Psychopharmacologia* **28**, 35 (1973).
7. B. G. Henriksson and T. Jarbe, *J. Pharm. Pharmacol.* **23**, 457 (1971).
8. J. H. Jaffe, in *The Pharmacological Basis of Therapeutics*, L. S. Goodman and A. Gilman, Eds. (Macmillan, New York, ed. 3, 1965), pp. 285–311.
9. J. Karkalas and H. Lal, *Int. Pharmacopsychiat.* **8**, 248 (1973).
10. G. Granutsos, R. B. Drawbaugh, M. D. Hynes, H. Lal, *Life Sci.* **14**, 887 (1974).
11. W. Bromberg, *Am. J. Psychiat.* **91**, 303 (1934); E. G. Williams, C. K. Himmelsbach, A. Wikler, D. C. Ruble, B. N. Lloyd, *Public Health Rep.* **61**, 1059 (1946).
12. M. Perez-Reves, M. C. Timmons, M. E. Wall, *Arch. Gen. Psychiat.* **31**, 89 (1974).
13. Supported in part by NIMH grant MH-04669. We gratefully acknowledge supplies of THC furnished by the Drug Abuse Research Advisory Committee of the Food and Drug Administration and National Institute of Drug Abuse.

29 July 1974; revised 17 September 1974

Primitively Columellaless Pollen: A New Concept in the Evolutionary Morphology of Angiosperms

Abstract. *Comparative study of pollen of the ranalean complex has revealed a remarkable, hitherto unrecognized characteristic of primitive angiosperm pollen, namely, its complete lack of columellae. Pollen with such exine has been designated* atectate *and taxa in the Magnoliaceae, Degeneriaceae, Eupomatiaceae, Annonaceae, and possibly Himantandraceae and Nymphaeaceae have pollen which is considered to be primitively columellaless.*

The pollen wall of most angiosperm pollen grains consists of two fundamentally different layers: an inner, more or less uniform cellulosic layer known as the intine, which is usually destroyed on acetolysis (*1*), and an outer, generally much more complicated and taxonomically useful acetolysis-resistant layer, the exine, which is composed of oxidative polymers of carotenoids or carotenoid esters, or both, known as sporopollenin (*2*). Recently, Van Campo (*3*) pointed out that the presence of distinct, well-defined, internal, upright, rodlike elements known as columellae, covered by a rooflike layer or tectum (tectate-columellate pollen, Fig. 1A) rather than a honeycomb-like network or an irregular spongy layer (alveolate pollen, Fig. 1B), is one of the main features in which the exine of angiosperm pollen grains differs in general from that of gymnosperms.

However, comparative study of pollen of the ranalean complex has revealed that the pollen exine in some of the most primitive families of angiosperms is structurally amorphous and devoid of any trace of either a columellate or alveolate structure (Fig. 1, C and D). Moreover, study of character correlation within the ranalean complex of primitive angiosperms strongly favors recognition of a major evolutionary trend; this goes from pollen with

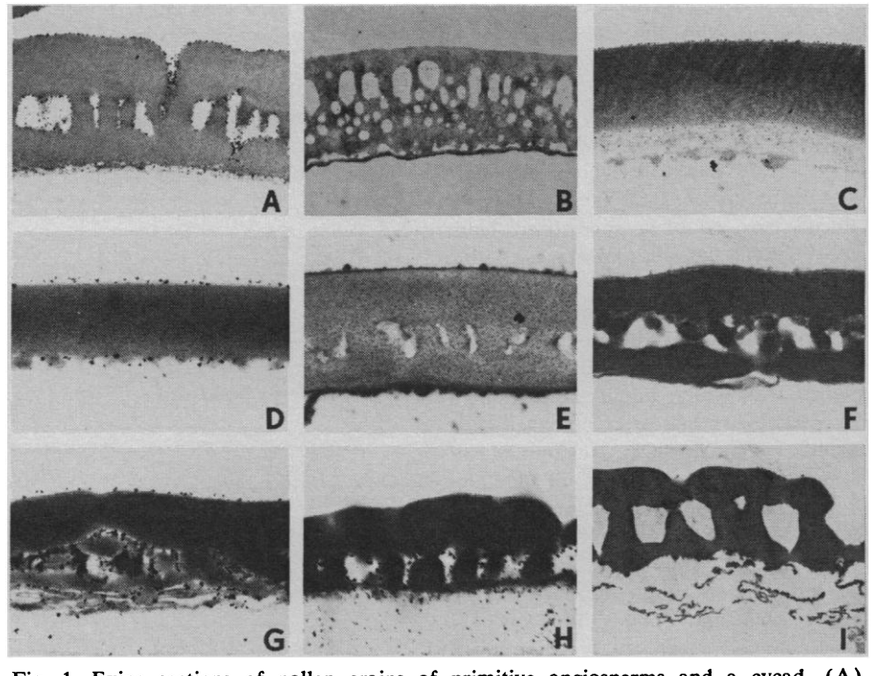

Fig. 1. Exine sections of pollen grains of primitive angiosperms and a cycad. (A) *Calycanthus floridus* L. (Calycanthaceae), with tectate-columellate pollen with well-developed columellae (× 9,425). (B) *Cycas revoluta* Thunb. (Cycadaceae), with aleveolate pollen (× 7,000). (C) *Degeneria vitensis* I. W. Bailey & A. C. Smith (Degeneriaceae), with atectate pollen (× 13,000). (D) *Eupomatia laurina* R. Br. (Eupomatiaceae), with atectate pollen (× 24,375). (E) *Aromadendron elegans* Blume (Magnoliaceae), with granular pollen with presumed incipient columellae (× 18,200). (F) *Magnolia fraseri* Walt. (Magnoliaceae), with granular pollen with presumed incipient columellae (× 14,950). (G) *Uvariastrum zenkeri* Engler & Diels (Annonaceae), with granular pollen (× 13,300). (H) *Desmopsis bibracteata* (Robinson) Saff. (Annonaceae), with granular pollen with presumed incipient columellae (× 19,475). (I) *Asimina pygmaea* (Bartr.) Dunal (Annonaceae), with pollen with well-developed columellae (× 3,795).

such homogeneous exine to pollen with what appear to be incipient, rudimentary columellae to pollen with well-developed columellae, and not the reverse. Thus, pollen grains of some extant flowering plants seem to be primitively columellaless, which is a new concept in the evolutionary morphology of the angiosperms.

While a number of flowering plants, such as certain aquatics, parasites, saprophytes, and so forth, have secondarily lost the columellate structure of their pollen grains (*4*), pollen which occurs in the ranalean families mentioned below is considered to be primitively columellaless, rather than reduced, for the following reasons. First, the order Magnoliales (*5*), to which most of the taxa with columellaless pollen belong, represents the most primitive order of living angiosperms when the totality of its characters is considered (*6*). Moreover, columellaless pollen characterizes the most primitive taxa within the Magnoliales, that is, certain Magnoliaceae, the Degeneriaceae, the Eupomatiaceae, and primitive members of the Annonaceae, while advanced magnolialean taxa, such as more specialized members of the Annonaceae, the Canellaceae, and the Myristicaceae, largely possess columellate pollen. Second, genera like *Degeneria* and *Eupomatia* are not submerged aquatics, parasites, or saprophytes, and therefore their pollen cannot be interpreted as having been reduced because of a specialized habit or habitat. On the contrary, pollen grains of most of the ranalean genera considered to be primitively columellaless have relatively thick exine with no indication of a general reduction in the pollen wall which might have resulted in a secondary loss of columellae. Most significantly, however, examples of stages in the evolution of tectate-columellate pollen may actually be observed within the ranalean families Magnoliaceae and Annonaceae.

Comparative study of pollen of the entire ranalean complex (*7*) suggests that columellae evolved in angiosperm pollen grains in the following manner. In fact, all of the evolutionary stages outlined below may be observed in pollen of the order Magnoliales. The most primitive type of pollen wall structure in angiosperms apparently is represented by the essentially homogeneous exine found in pollen grains of genera such as *Degeneria* (Fig. 1C) and *Eupomatia* (Fig. 1D) (*8*). The term *atectate* has been chosen to describe pollen grains such as these, which lack a tectum because their more or less amorphous exine has little or no internal structure. As previously indicated by Dahl and Rowley (*9*), pollen grains of *Degeneria* exhibit a remarkable degree of psilateness. Such pronounced lack of sculpturing seems to be characteristic of most atectate pollen, although some atectate pollen grains possess surface details, most commonly in the form of foveolae (pits). From structureless, atectate pollen, pollen grains which may be loosely termed granular appear to have evolved. Such granular pollen is found in both the Magnoliaceae (Fig. 1, E and F) and the Annonaceae (Fig. 1, G and H). The granular layer in pollen of this type apparently may occur either in the middle of the exine, through development of enclosed, internal cavities within the exine (Fig. 1E), or on its interior surface, due to development of a zone of more or less spherical granules on the exine's inner face (Fig. 1, G and H). Granular pollen seems to lead to a more or less unstabilized stage characterized by development of what appear to be incipient columellae (Fig. 1, F and H). The culmination of this trend in exine structure is apparently reached with the evolution of well-developed columellae, either by enlargement and stabilization of intraexinal cavities resulting in a well-defined columellate layer, or by fusion of granules to form a basal nexine layer and well-marked columellae (Fig. 1I), which may reach 15 μm in length in some specialized members of the Annonaceae (*10*).

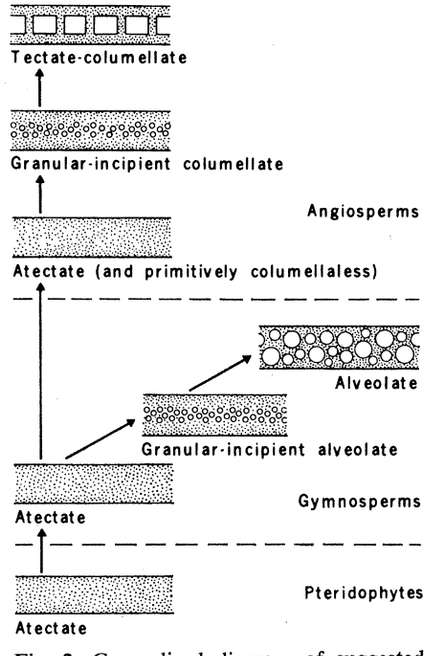

Fig. 2. Generalized diagram of suggested major evolutionary trends in spore-pollen exine structure of vascular plants. See text for discussion.

Although light microscopy shows the pollen wall of the monotypic magnolialean family Himantandraceae to be more or less homogeneous, at this time we can only infer that it is indeed primitively columellaless since the exine in *Galbulimima* (=*Himantandra*) is itself reduced and we do not presently have transmission electron micrographs of pollen of this genus. In addition, some nymphaeaceous pollen grains which seem to be more or less homogeneous or granular (*11*) may be primitively columellaless also, but more investigation of palynological trends within the family Nymphaeaceae is required to confirm this.

Our studies further suggest that columellae have evolved independently a number of times, even within, for example, different subfamilies of the Annonaceae. Moreover, the phylogenetic position of genera with atectate pollen within the family Annonaceae agrees well with the relative advancement of annonaceous genera previously proposed on the basis of other characters (*12*). Finally, it seems certain that columellae evolved within the exine itself, and were not originally situated externally on its surface. Evolutionary trends within primitive angiosperms with columellate pollen also support this conclusion, inasmuch as tectate pollen with internal columellae is clearly more primitive than semitectate pollen with an open reticulum, or intectate pollen with no tectum and exposed, external columellae (*13*).

While some authors have compared the granular pollen exine observed in some gymnosperms and the granular structure which occurs in the pollen of certain angiosperms (*14*), we consider it likely that the alveolate structure of gymnosperm pollen grains and the columellate structure of angiosperm pollen have evolved independently from atectate, structureless pollen via a more or less similar granular stage, since pollen grains of some of the most primitive of living angiosperms, such as *Degeneria* and *Eupomatia*, are structurally amorphous and essentially show no trace of a granular exine structure. Moreover, granular pollen itself may have led to the development of two different kinds of alveolate exine structure in gymnosperms, namely, pollen with an irregular, spongy layer, such

as occurs in some coniferophytes, particularly in the saccate pollen of the Pinaceae (*15*), and pollen with a more organized, honeycomb-like network, which may be observed in members of the cycadophyte line of gymnosperms (*16*). Significantly, spores of pteridophytes such as *Lycopodium, Psilotum,* and *Archaeopteris* fundamentally have structureless spore walls (*17*) and therefore may be considered to have a basically atectate sporoderm. Figure 2 summarizes the main evolutionary trends in spore-pollen exine structure of vascular plants which we consider most likely on the basis of current data.

JAMES W. WALKER
Department of Botany, University of Massachusetts, Amherst 01002

JOHN J. SKVARLA
Department of Botany and Microbiology, University of Oklahoma, Norman 73069

References and Notes

1. K. Faegri and J. Iversen, *Textbook of Pollen Analysis* (Hafner, New York, ed. 2, 1964), p. 15.
2. G. Shaw, in *Sporopollenin*, J. Brooks, P. R. Grant, M. Muir, P. van Gijzel, G. Shaw, Eds. (Academic Press, London, 1971), p. 305.
3. M. Van Campo, *C. R. Hebd. Seances Acad. Sci. Ser. D Sci. Nat.* **272**, 2071 (1971).
4. S. Nilsson and J. J. Skvarla, *Ann. Mo. Bot. Gard.* **56**, 420 (1969).
5. The order Magnoliales as used here is considered to include the following seven families: Magnoliaceae, Degeneriaceae, Himantandraceae, Eupomatiaceae, Annonaceae, Canellaceae, and Myristicaceae.
6. A. Cronquist, *The Evolution and Classification of Flowering Plants* (Houghton Mifflin, Boston, 1968); A. Takhtajan, *Flowering Plants: Origin and Dispersal* (Smithsonian Institution Press, Washington, D.C., 1969); R. F. Thorne, *Aliso* **6** (No. 4), 57 (1968).
7. J. W. Walker, in *The Origin and Early Evolution of Angiosperms*, C. B. Beck, Ed. (Columbia Univ. Press, New York, in press).
8. While the inner layer of the exine of *Degeneria* pollen grains may be less dense than the outer layer, it is difficult at this time to decide whether this represents the very beginning of a granular-incipient columellate layer (see text) or a rudimentary endexine.
9. A. O. Dahl and J. R. Rowley, *J. Arnold Arbor. Harv. Univ.* **46**, 308 (1965).
10. J. W. Walker, *Science* **172**, 565 (1971).
11. F. Roland, *Pollen Spores* **7**, 5 (1965); J. Ueno, *J. Biol. Osaka City Univ.* **13**, 99 (1962); J. Ueno and S. Kitaguchi, *ibid.* **12**, 83 (1961).
12. J. W. Walker, *Contrib. Gray Herb. Harv. Univ.* **202**, 1 (1971); *Grana* **11**, 45 (1971); *Bot. J. Linn. Soc.* **65**, 173 (1972); *Taxon* **21**, 57 (1972). Earlier characterization by J.W.W. of pollen of some annonaceous genera as "microtectate" and "reduced" is now considered implausible in light of more extensive palynological study of all the families in the ranalean complex.
13. J. W. Walker, *Am. J. Bot.* **61**, 891 (1974).
14. M. Van Campo and B. Lugardon, *Pollen Spores* **15**, 171 (1973); A. Le Thomas and B. Lugardon, *C. R. Hebd. Seances Acad. Sci. Ser. D Sci. Nat.* **278**, 1187 (1974).
15. M. Van Campo and J. Sivak, *Pollen Spores* **14**, 115 (1972).
16. J.-C. Audran, *C. R. Hebd. Seances Acad. Sci. Ser. D Sci. Nat.* **270**, 1455 (1970); G. Erdtman, *Handbook of Palynology* (Hafner, New York, 1969); pp. 444–447; B. M. Gullvåg, *Grana Palynol.* **6**, 435 (1966); J. J. Skvarla and J. R. Rowley, *Am. J. Bot.* **57**, 519 (1970).
17. J. M. Pettitt, *Bull. Br. Mus. (Nat. Hist.) Geol.* **13**, 223 (1966).
18. Supported in part by NSF grant GB-35475, by faculty research and faculty growth grants from the research council of the University of Massachusetts, Amherst, to J.W.W., and by NSF grant GB-39858 to J.J.S.

12 August 1974

Growth Hormone: Independent Release of Big and Small Forms from Rat Pituitary in vitro

Abstract. *Sequential release of big and small forms of growth hormone by perifused rat pituitaries has been demonstrated by immunoprecipitation. The results suggest that either the two forms are independently synthesized and released, or that a newly synthesized molecule of big growth hormone follows one of two paths: direct release or intracellular processing through the storage compartment with conversion to small growth hormone.*

Heterogeneity of molecular size is characteristic of several protein and polypeptide hormones (*1*). Hormone forms that are larger than the monomeric molecule, and that serve as biosynthetic precursors, have been identified for insulin (*2, 3*), Parathormone (*4*), and glucagon (*5*). The earliest detectable biosynthetic form of pituitary growth hormone (GH) is a large molecular species, excluded from a G-200 Sephadex column. This large GH is associated with ribonucleic acid, and has been proposed to represent a complex of nascent GH associated with the GH polysome (*6*). In addition to that for GH (*7, 8*), large molecular species have also been shown for human placental lactogen (*9*) and prolactin (*7*).

Growth hormone forms of intermediate size in pituitary extracts (*10*) and plasma have been described. Two plasma forms, each designated as big GH, have been reported. One form exhibits an apparent molecular weight three times that of monomeric GH (*1*); and the other, twice that of the monomer (*11, 12*). Their relationships to the predominant monomeric form of GH are not clear. Experiments on the big GH that is twice the size of monomeric GH are described in this report. The relationship of this big GH and small (monomeric) GH was investigated in rat pituitary and incubation medium using an in vitro perifusion (*13*) system.

Rat adenohypophysial quarters were first incubated for 3 hours in Krebs Ringer bicarbonate buffer (KRB) supplemented with bovine serum albumin (1 percent) and glucose (150 mg/100 ml) and containing [^{14}C]leucine (5 μc/ml). This procedure established an intracellular, stored pool of [^{14}C]GH. Labeled explants were transferred to a 0.25-ml chamber and perifused at 0.2 ml/min by KRB without radioactive precursor. Fractions of 1 ml were collected until a constant basal release of stored [^{14}C]GH was achieved (*13*). The perifusion medium was then changed to KRB supplemented with [^{3}H]leucine (3 μc/ml), and the perifusion was continued for an additional 2 hours. Individual effluent fractions were dialyzed to remove excess radioactive amino acid and were immunoabsorbed with normal guinea pig serum and with goat antiserum against guinea pig serum to reduce nonspecific background radioactivity. They were then reacted with excess specific monkey antiserum against rat GH (individual sample blanks were reacted with normal monkey serum) and precipitated with goat antiserum against monkey gamma globulin (*8, 14*). Washed precipitates were solubilized, counted, and analyzed by computer for [^{3}H]GH and [^{14}C]GH (*6*).

Accumulation of [^{14}C]GH in the perifusion effluent was expressed as percentage of the total [^{14}C]GH in the explant at the time of each collection interval (*13*). Total [^{14}C]GH was calculated by subtracting released [^{14}C]-GH from total [^{14}C]GH present at the onset of the perifusion. When expressed in this way, [^{14}C]GH accumulation in the effluent was linear once explant equilibration had occurred (Fig. 1). Release of [^{3}H]GH began immediately after explant exposure to [^{3}H]leucine, and its accumulation in medium was linear for approximately 1 hour. After 1 hour, [^{3}H]GH release remained linear but the rate was increased. The origin of released [^{14}C]GH was the cellular storage compartment, since new synthesis of [^{14}C]GH had ceased after transfer to the perifusion chamber (*13*).

Approximately 40 to 60 minutes are required for transfer of a newly synthesized GH molecule from the synthesis mechanism to its release in vitro