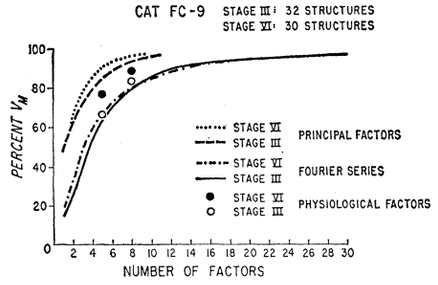

Fig. 1. Variance (V_m), plotted against the number of factors (abscissa) obtained from analyses by principal factors, physiological factors, and Fourier series. At stage III a conditioned avoidance response to the onset of steady light has been established, and the flicker is a neutral stimulus; at stage VI, a differentiated avoidance response has been established between two frequencies of flickering light.

dian, mesencephalic reticular formation, and the dorsal hippocampus. In three additional cats, some indication has been obtained that these relationships change during spontaneous errors or during blockade of performance after the administration of reserpine (*7*).

The reconstruction factors provided by the method of principal factors have no physiological significance but simply permit a concise and convenient representation of the waveforms. In further work, a reconstruction scheme based on factors chosen by physiological criteria was utilized. Selection of waveforms showing the highest loading on the first few principal factors allows the construction of a set of oblique physiological factors. Regression equations can now be calculated which describe the waveshape in many regions of the brain as linear combinations of the waveshapes in a small number of anatomical loci. Examination of such physiological regression equations suggests that as a stimulus acquires a particular meaning, the functional influence of the thalamic reticular formation is markedly enhanced. Table 2 presents regression equations which reconstruct, in terms of physiological factors, the same set of waveshapes for which a principal factor description is provided in Table 1. Details of the reconstitution of waveforms with these two techniques have been discussed (*7, 8*).

It is of interest to compare the degree of data reduction obtained from analyses by principal factors and physiological factors to that obtained from an analysis by Fourier series. A comparison can be made from the data in Fig. 1 where the variance (V_m) of the signal space accounted for by m factors is plotted against m for each of the three methods. By this measure, the analyses by both principal factors and physiological factors are more efficient than by Fourier series. Some degree of similarity of waveshape is to be expected due to constraints on the frequency response of the potentials. However, the greater efficiency of the analyses by principal factors and physiological factors suggests that some of the similarity is due to covariation of certain clusters of terms in the set of waveforms as described by Fourier series. This covariation may be due to functional relationships that exist between the anatomical sites from which the potentials were recorded.

As informational significance is attached to a stimulus by conditioning, the average potentials evoked by that stimulus in diverse brain regions acquire a marked similarity in waveshape. The observed correspondence between the time course of electrical activity in diverse brain regions, and the diminution in this correspondence with inappropriate performance, suggests that this reflects the organization of a functional system processing information about the stimulus. The data reported here are in support of the comparator hypothesis stated earlier. The observed waveshape correspondence between neural regions is interpreted to mean that the pattern of axonal discharges impinging as afferent input upon a region produces in that region a reflection of the macropotential waveshape in the region whence the axonal discharges originate. Changes in waveshape correspondence between regions are interpreted as changes in the effectiveness of the influence of certain regions upon others. The data analysis and reduction techniques outlined herein are applicable to a wide variety of other kinds of electrophysiological and neuropharmacological problems. A fuller account of these methods and of the result obtained has been presented recently and will shortly be published (*7, 8; 9*).

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17 June 1963

Ginkgophyton (Psygmophyllum) with a Stem of Gymnospermic Structure

Abstract. *A specimen identified as* Ginkgophyton (Psygmophyllum) *with a structurally preserved stem of gymnospermic organization has been collected from a late Devonian stratum in New York.*

The taxonomic position of a complex of Paleozoic genera consisting of flabelliform or cuneiform leaves, occasionally attached to branching axes, has been one of the most perplexing of paleobotanical problems for nearly a century. Included in this group, which ranges stratigraphically from Devonian to Permian, are *Platyphyllum, Germanophyton, Ginkgophyllum, Ginkgophyton, Psygmophyllum,* and, indeed, *Enigmophyton.*

In the most recent discussion of these genera, Høeg (*1*) provides an excellent summary of their morphology and an analysis of nomenclatural problems. As he emphasizes, *Ginkgophyllum* and *Psygmophyllum* were established for Upper Carboniferous and Permian species of distinctive morphology. Both, but especially *Psygmophyllum*, have also been used for the inclusion of Devonian species. Of the other genera, all of which are characteristically Devonian, *Enigmophyton* Høeg is the most completely known. *Enigmophyton superbum,* from the Upper Middle or Lower Upper Devonian of Spitsbergen, is reported to have a dichotomizing branch system bearing a secondary system of smaller lateral branches and sessile, flabelliform, dichotomously veined leaves up to 16 cm long and 12 cm wide (at the broadest point). These

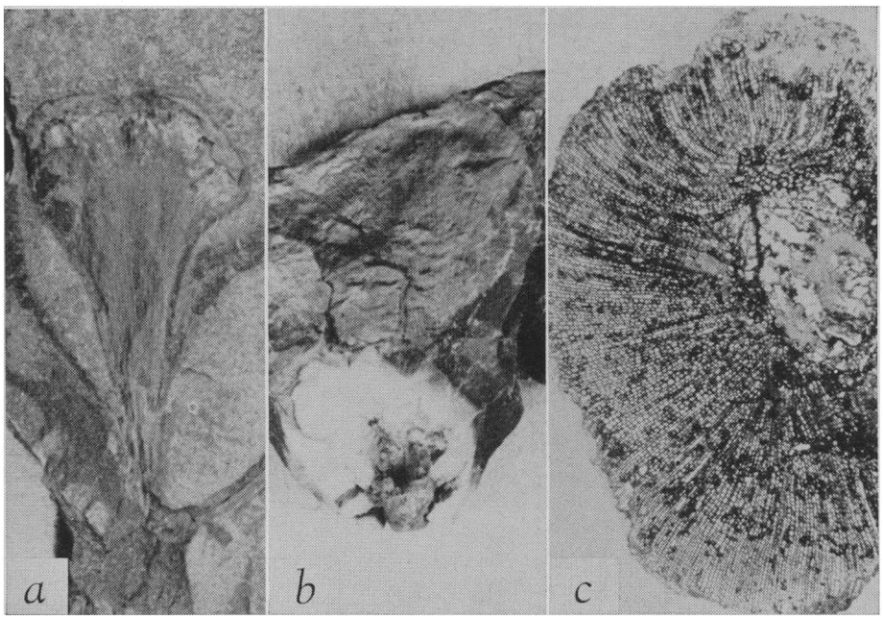

Fig. 1. *Ginkgophyton* sp. (*a*) Comparatively complete leaf, but with the narrow basal part incompletely preserved; (*b*) structurally preserved axis bearing leaves. The axis is oriented at an angle of approximately 80° to the bedding plane; (*c*) transverse section of a segment of the axis shown in *b* (*a* and *b*, natural size; *c*, × 20).

simple leaves occur at or near points of bifurcation. Apparently the only important known difference between *Enigmophyton* and *Ginkgophyton* is the absence of dichotomous branching in the latter. The leaves of *Ginkgophyton* are thought to possess sheathing bases, whereas the mode of attachment in *Enigmophyton* is not known.

Fructifications found in association, but not in organic connection, with *E. superbum* are thought by Høeg to have been borne on this plant at the positions of the short lateral branches. The fructifications consist of a dichotomous branch system, the terminal parts of which bear laminate sporophylls of broadly ovate form, each with a single adaxial sporangium. Megaspores and microspores have been isolated from different sporangia of the same fructification.

Because of the thin expansive leaves of the species in this complex and, for many years, a lack of positive evidence of vascular tissue, the group has been thought by some to be algal, or at least aquatic. This view seemed to be substantiated by *Germanophyton psygmophylloides* which, because of a structure believed to consist of tubes, was assigned (*2*) originally to *Prototaxites,* a form considered by many to be the axis of a large marine alga.

On the other hand, Høeg (*1*) has noted the similarity of the leaves and, especially, the fructifications associated with *Enigmophyton superbum* to certain lycopsoid plants such as *Barrandeina,* and he is inclined to believe that it was a vascular cryptogam.

The leaf shape and form as well as the dichotomous venation suggest plants such as *Cordaites* or some of the ginkgophytes as indicated by the names *Ginkgophyton* and *Ginkgophyllum.* No one, however, in recent times has seriously suggested close affinity of any of the Devonian forms with these groups since *Cordaites* is not positively known earlier than Upper Carboniferous, and ginkgophytes are considered to have arisen first in the Permian. There is, furthermore, no convincing published evidence of true seeds in the Devonian. The assignment of certain genera by earlier workers to such diverse groups as the ferns, cycads, and palms has added further to the confusion which has surrounded this group.

Some of the questions about this interesting and perplexing group of plants may now be answered in part, because of the discovery of a specimen identified as *Ginkgophyton* which consists of leaves in organic connection with a structurally preserved axis (Fig. 1*b*). The specimen, oriented across the bedding plane of a siltstone, was collected recently from Lower Upper Devonian beds at Pond Eddy, Sullivan County, New York. It is a branching axis, bearing spirally arranged leaves, which conforms to the definition of *Psygmophyllum* sensu Arbor (*3*), that is, *Ginkgophyton* (*1*), but the leaves are only about one-half as large as those of specimens usually attributed to the genus. Associated with this specimen are several compressed axes with attached leaves, as well as some isolated leaves (Fig. 1*a*).

The largest leaf thus far observed (only partially complete) is 7 cm long. Although there appears to be considerable variation in size, the extent of this variation will be difficult to determine because of the fragmentary nature of the leaves. I do not believe, however, that any leaf, if complete, would exceed 10 cm in length.

In internal structure (Fig. 1*c*) the stem consists of a pith, esentially triangular in transverse section, containing peripheral strands of primary xylem of irregular transverse shape. This is surrounded by a thick layer of secondary xylem consisting of narrow rays and tracheids bearing alternate circular-bordered pits. The leaf trace originates as a single strand that branches from a mass of primary xylem extending outward into the surrounding secondary xylem prior to separation of the trace.

As yet no sections have been made which show the nature of the trace at later stages. It seems possible, however, that certain parts of the specimen will reveal preserved phloem, cortex, and, hopefully, leaf bases which will provide other important details of structure.

It can be stated positively that this specimen represents a terrestrial vascular plant, not an alga. On the basis of the information at hand, the axis of this plant seems to be quite similar to the pycnoxylic members of the Calamopityeae such as *Eristophyton, Bilignea,* and *Endoxylon.* The great similarity of these genera to the Cordaitales has been emphasized (*4*). Except for the narrow rays, the new specimen is also similar to *Calamopitys* and *Stenomyelon muratum.*

In external morphology the similarity in leaf form and venation to *Cordaites* should again be noted. Furthermore, the leaves exhibit an interesting resemblance to the large flabelliform pinnules of *Archaeopteris obtusa.*

I believe that the plant represented by this specimen is, like *Archaeopteris* (*5*), one of the pteridophytic progymnosperms which flourished during late Devonian times (*6*).

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Plaque Assay for Measurement of Cells Infected with Zoster Virus

The isolation of viruses belonging to the varicella-zoster group in human cells by Weller and his associates (*1*, *2*) led to attempts to study the properties of these viruses by conventional methods. However, viruses in this group have proved to be difficult to grow and can be propagated only when intact infected cells are used as inoculum. A recent isolate of zoster virus (strain EY) in our laboratory has the same characteristics as the strains previously isolated by others. The virus was isolated in human embryonic lung fibroblasts from fresh vesicular fluid from a patient with generalized zoster infection. Many attempts to detect cell-free virus in the extracellular fluids were negative. This was true even when the cultures were actively deteriorating. The virus has therefore been passed by adding dispersed cells from infected cultures to cell suspensions or monolayers of human embryonic lung cells. Stocks of virus are maintained by freezing infected cells in Eagle's medium with 20-percent calf serum and 15-percent glycerol in liquid nitrogen at −195°C, under which condition the virus-cell complex retains its infectivity.

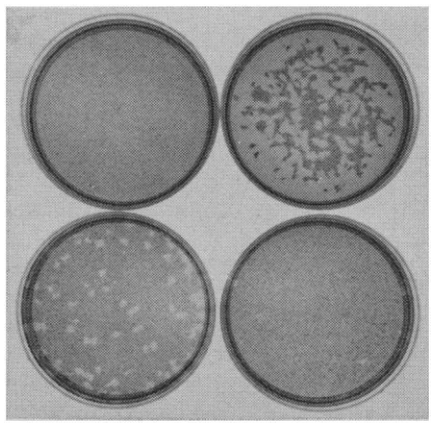

Fig. 1. Plaques obtained under an agar overlay 10 days after inoculation of human embryonic lung fibroblasts with cells infected with zoster virus. (Upper left) uninoculated. (Upper right) dilution of 10^{-2}. (Lower left) dilution of 10^{-3}. (Lower right) dilution of 10^{-4}. (× 0.4)

Infected cultures exhibit focal cytopathic effects which become manifest throughout the culture. This spread of the effect is slower than that exhibited in human embryonic lung cells by herpes simplex virus (24 to 48 hours to complete degeneration) but more rapid than that seen with cytomegalovirus (5 to 6 days) when tested under the same conditions.

Another feature that distinguishes this isolate from cytomegalovirus and herpes simplex virus is its lack of viability when infected cells are disrupted. Thus, freezing and thawing or sonic oscillation of infected cells causes complete destruction of the infectivity of the virus. Herpes simplex virus survives both these procedures, and we have found that cytomegalovirus can be liberated from infected cells by sonic oscillation.

Serums from convalescent patients with zoster or varicella infection react with virus antigen produced in these cells as measured by immunofluorescence, but specific herpes simplex antiserums prepared in rabbits fail to do so. Serums from very young children without previous exposure to varicella often react with cytomegaloantigen but do not react with zoster antigen.

During the course of these studies, it was noted that cultures inoculated with small numbers of infected cells exhibited distinct circumscribed areas of degeneration with clear centers. This has also been described by Weller *et al.* (*2*) and by Taylor-Robinson (*3*). Attempts to obtain a quantitative plaque assay without an overlay, however, were unsuccessful because of the rapid spread of the infection under the fluid medium; this was probably mediated by detached cells. An attempt was therefore made to develop a plaque assay system for cells infected with zoster virus under a semisolid overlay. Infected human fibroblasts in which 50 to 75 percent of the cells showed some measure of cytopathic effect were trypsinized with 0.2 percent trypsin, resuspended in growth medium consisting of 90-percent Eagle's medium and 10-percent calf serum, and adjusted to a concentration of 2×10^7 cells per milliliter. The cells were then diluted in nutrient medium and plated out in either 0.5 or 1.0 $\log_{10}$ dilutions on monolayers of human fibroblasts growing in 60-mm petri dishes.

Table 1. Plaque assay of cells infected with zoster virus; read 10 days after inoculation when plaques ranged from 2 to 3 mm in diameter. PFC, plaque-forming cells.

Dilution	Plaque counts	Av.	PFC per ml
10^{-3}	*		
10^{-4}	33, 36, 36, 42	36.3	3.6×10^6
10^{-5}	4, 2, 2, 7	3.8	3.8×10^6
$10^{-3.5}$	*		
10^{-4}	58, 60	59	5.9×10^6
$10^{-4.5}$	26, 22	24	7.6×10^6
10^{-5}	6, 6	6	6.0×10^6

* Too numerous to count in two to four plates used at dilution indicated.

After an adsorption period that varied from 3 to 18 hours, the nutrient fluid was withdrawn and an overlay that consisted of either 1 percent agar or 2 percent methyl cellulose (4000 cp) in Eagle's basal medium plus 10-percent calf serum was added to the petri dishes. The cultures were then incubated at 37°C in an atmosphere of 5-percent CO_2 for varying periods of time. The day before plaques were to be examined, 3 ml of a 1 : 7500 concentration of neutral red was added to the cultures and the cultures were reincubated overnight at 37°C in 5-percent CO_2.

Plaques developed between 4 and 7 days after inoculation of the cells.

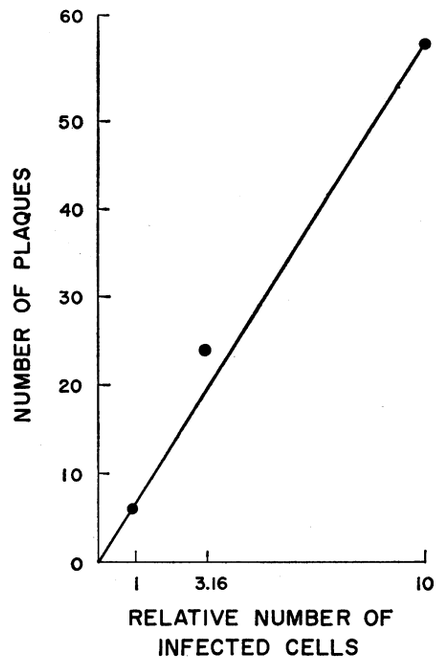

Fig. 2. Linear relationship between number of cells infected with zoster virus on monolayers of human embryonic lung fibroblasts and plaques obtained under an agar overlay 10 days after infection.