THE MATERIALS AND METHODS OF PALAEOBOTANY

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INTRODUCTION

UNDER the guidance of the late Prof. Birbal Sahni, the plant-bearing rocks of India were subjected to scrutiny by every technique known to the palaeobotanist. The most unpromising and controversial sediments were investigated with complete freedom from prejudice (SAHNI, 1941, 1946, 1948; SITHOLEY, 1946). In memory of our lamented colleague, I have chosen as the subject of this contribution, *The Materials and Methods of Palaeobotany*. New techniques — and improvements of old techniques — have not only wrought a revolution in the interpretation of fossil plants but also have provided methods for investigating sediments hitherto considered unworthy of attention.

Some years ago while turning the pages of a venerable tome *History of Egyptian Mummies* by Dr. Thomas Pettigrew (1833), I stumbled upon a discovery made more than a century before, that bituminization may, under certain circumstances, preserve animal tissues indefinitely. The similarity between embalming with bitumens to coalification or bituminization of plant tissues convinced me that a vast opportunity to investigate fossil plants with greater accuracy than heretofore merely awaits the improvement of known techniques or the invention of new ones.

During the past thirty years palaeobotany has tended to fragment into several specialisms, each with its own methodology and tradition. Although this is the inevitable result of activity and progress, in such fragmentation there is a loss of coherence. Taxonomic systems become inordinately artificial, terminologies grow complex and the philosophy of the science becomes confused.

Palaeobotany, however narrowly or inclusively we may wish to define it, is the study of fossil plants. This is the only common denominator in our chosen field of research. Yet we have contributed to the separation of the specialisms by disregarding the nature of fossils. A proper correlation between form and structure would minimize the impossible, even ridiculous, duplication of names, creation of artificial form genera, and establishment of purely arbitrary systems of nomenclature.

Consider but one example of this tendency, Palaeozoic spores and pollens, more precisely called palynology. In utter disregard of parent sporangia, taxonomic group or biological origin, the multitudinous forms are binomially identified, classified, and interpreted. Gradually we are erecting a colossus of meaningless terms which will haunt our synonymies for years to come. The pragmatic or empirical temporary advantage of a workable system for coal correlation and similar purposes is of little long-range importance. Existing techniques are sufficiently refined and perfected to determine within the space of a few years the biological identities of hundreds, perhaps a thousand, Palaeozoic spores and pollens.

The changing views of petrifaction merely reflect a growing realization of the relative indestructibility of organic materials (DARRAH, 1941). The *ex cathedra* pronouncement "too delicate for preservation" has passed unchallenged for a century. No greater obstacle has impeded exploration of new techniques, new approaches to old problems, and pioneering in new problems.

Dr. Pettigrew in 1833 was examining the heart of an Egyptian mummy estimated to be 3000 years old. He had removed the bituminous embalming preservatives by maceration of the heart in "spirits". Within a few days the softened tissues began to decompose and in a week putrefied. The chemical composition of the macerated heart was such that normal putrefaction occurred promptly. On several occasions I have observed thriving colonies of yeasts and molds growing on macerated residues of bituminous coals but in these cases the polysaccharides and "humic" materials of the coals had been fundamentally altered by prolonged chemical processing.

In order to repeat Pettigrew's observation I sought the co-operation of Dr. Ernest Hootan and Dr. Donald Scott of the Peabody

Museum of Harvard University. These gentlemen provided me with tissues of a female Egyptian mummy and dry mummy Arizona Indian, the former being of the 21st Dynasty *ca.* 1000 B.C. and the latter *ca.* A.D. 1000. Portions of the pterygoid muscle from the oral cavity of the Egyptian mummy were macerated by three methods : mineral acids and alcohol, benzene and petroleum ether. Well-preserved tissues were recovered by all methods. On the other hand, no recognizable tissues were recovered from the dry mummy, which incidentally was not impregnated with bituminous substances but rather was completely desiccated.

The preserving qualities of bituminization are well known. For instance, Brøgger in preparing wooden objects found in the Oseberg ship used the following technique : wood steamed and soaked with creosote followed by a bath in linseed oil in which carbolineum (an anthracene oil) is dispersed. Soft woods were hardened with alum. This procedure simulates a common type of fossilization.

These examples draw attention to the fact that normally destructible tissues *have been* preserved by artifactual bituminization and that tissues *can be deliberately* preserved by bituminization.

MACERATION TECHNIQUES

More than a century ago Goeppert successfully removed the petrifying substance of silicified and calcified specimens recovering in these experiments organic residues of the original plant bodies. In several cases he observed the cellulose reaction with sulphuric acid. Goeppert (1864) performed many macerating experiments and derived from these investigations a better understanding of the nature of fossils than any other palaeobotanist of the 19th century. As with most prophets, his opinions were generally unappreciated.

Other maceration techniques were developed by several investigators of whom Schultz and Gümbel deserve special mention. Schultz in 1855 devised the mixture of mineral acids (which still bears his name) for macerating low rank coals. Subsequent alkalination was accomplished with sodium hydroxide. Caustic soda tended to cause separation of cell masses and, if concentrated, dissolved certain constituents. Gümbel minimized this disintegration by gentle alkalination with ethyl alcohol.

Briefly then by 1850, the occurrence of embedded plant bodies was not only recognized, but also fairly generally understood. It was known also that resistant cutinized parts could be recovered from lignitic and bituminous coals.

Solms-Laubach (1891, pp. 2-13) distinguished two types of mineral petrifaction, the first in which there is no organic residue (i.e. all apparent structure is pseudomorphic), and the second, embedded fossils. This distinction, which today is recognized as an over-simplification, epitomized the results of Goeppert's extensive investigations.

All these chemical methods involve the dissolution of inorganic materials and the degradation of organic residues. The degree of chemical alteration resulting from such treatment has long been in dispute and, except for certain simple and obvious reactions, no conclusive evidence has ever been presented. Laboratory experiments on the degradation of cellulose and lignin offer little direct evidence on this question because they do not duplicate natural conditions in which the organic materials are already degraded. Direct observations on the individual constituents of naturally occurring sediments are extremely meagre.

For many years the thin ground section has been respected as the ideal preparation because, by its very nature, it portrays the true condition of the fossil structure. Any technique which tampers with the matrix or modifies the plant, no matter how little, is viewed with suspicion. There is, to be sure, a measure of truth in this assumption. Certain thin sections, as of the Rhynie chert, the Black Hills cycadeoids and the Permian ferns of Chemnitz, exhibit magnificent preservation. Fungal sporangia, mycelia, stomata, rhizoids and even cell contents may be recognized. Yet a silica petrifaction is oftimes disappointing. Many woods from the Triassic of Arizona and the Cretaceous of Wyoming preserve nothing of interest, indicating scarcely any features except gross form, growth rings, and scattered cell masses. Such petrifactions are little more than pseudomorphs.

By 1880, despite the work of Goeppert, it was generally assumed that the silicified (or calcified) petrifaction was in reality molecular mineral replacement and the structure purely pseudomorphic. Chemical analyses disclosed insignificant carbon content, a fact which was interpreted as proof of the disappearance of

the plant body. The possibility that the original structure could be present while not denied, was ignored. The first break in this long-standing prejudice was the demonstration by Marie Stopes that relatively large masses of original tissue could be recovered by demineralization.

Indeed, Stopes (1918) had succeeded in demineralizing completely a specimen of silicified wood recovering the tssues in an excellent state of preservation. It was obvious that so-called mineral molecular replacement had not occurred but rather the wood was embedded in silica, essentially as Goeppert had claimed. How prevalent this form of silica petrifaction is has not yet been determined, but I have been able to recover recognizable plant tissues from macerations of Rhynie chert, and Triassic silicified woods, and from several hundred specimens of calcareous and pyritized coal balls.

In this connection it is noteworthy that certain cherts macerated for microfossils have yielded algae. The possibilities inherent in early Palaeozoic sediments have scarcely been considered.

Maceration techniques, of course, have been employed in the investigation of coals for many years. Subjection of bituminous coals to maceration by a mixture of hydrochloric and nitric acids (to which crystals of potassium chlorate are sometimes added) has revealed the presence of many identifiable materials such as spores, pollens, cuticles, mycelia, and relatively large masses of discrete tissues. The process of coalification, like petrifaction, has proved to be extremely complicated.

The application of this maceration technique to " coalified " compressions was a logical refinement in technique. One of the earliest attempts to recover pollen from a so-called impression was Sellards' experiment with *Codonotheca* (1903). Since that date, similar methods have been widely used so that today a large percentage of promising fructifications which reach the hands of researchers are tested for spores or pollens by some variation of the basic maceration technique. Mention of only a few examples will indicate the importance of this procedure : Hartung (1933) on the calamarians, Harris (1939) on Triassic bryophytes, Florin (1933) on the conifers and cordaites, Sahni (1923) on *Glossopteris*, and Arnold (1936) on the Devonian *Archaeopteris*. Typical of the structures which can be recovered by this method are the spores shown in Figs. 1 and 2.

TRANSFER TECHNIQUES

It has been assumed generally that such cutinized or resinoid structures as the exospore of spores and pollens, cuticles, and heavily lignified xylem elements are so resistant to hydrolysis and chemical attack by mineral and organic acids that their frequence and fidelity of preservation are greater than other types of tissue. Although this is relatively true, the assumption tends to obscure the occurrence, indeed the prevalence, of more delicate structures. The possibility that a whole mount or " transfer " of a bleached or cleared structure might provide much more information than macerated residues was ingeniously explored by Walton (1923). The results exceeded expectations.

The Walton technique in basic terms involves four steps :

1. Affixing the specimen, using balsam as the adhesive, to a glass slide.
2. Coating the exposed glass with wax.
3. Macerating the matrix with mineral acids, usually hydrofluoric, to remove the associated rock.
4. Washing the specimen thoroughly.

By way of final preparation the specimen is dehydrated, covered with balsam, and protected permanently by a cover glass. Thus the specimen is " transferred " from the rock to a glass slide or plate.

There are two limitations to this method, the specimen is generally dense and opaque, and further manipulation is difficult, if not impossible. The limitation due to opacity may be overcome to some extent by photographing with infra-red light (LECLERCQ, 1933).

The transfer technique was a notable contribution to the methodology of palaeobotany because it demonstrated, beyond dispute, that the so-called carbonized impression was in many cases a compression of the original plant body. Of equal significance was the proof that in compression lateral distortion is negligible. Thus freed from prejudice and preconception it was possible to turn to still greater refinements in technique.

In view of the achievements of maceration and transfer techniques, the recovery of mummified compressions with an intermediate transfer process is not a very great departure. If the bituminous impregnating substances were dissolved and the specimen loosened, the objective could be gained with a minimum of manipulation and destruction,

Various procedures more or less similar have proved to be successful. The following schedule is typical.

1. Slowly macerate the specimen in a solution of equal parts of nitric acid, hydrochloric acid and water. Maceration should continue from 3 to 7 days. Some types of matrix require longer periods and a change of fresh solution.

2. Wash the specimen gently in running water for 4 hours, or immerse in water for 6 hours with at least four changes of water.

3. At this stage the matrix should be tested for softness by means of a fine needle. If it has become soft or somewhat mushy and the specimen has loosened along the edges, it should be possible to lift the specimen gradually by prying gently around the periphery working a little at a time. If, on the other hand, the specimen does not yield, further maceration is desirable.

4. When the acid maceration and rinsing have been accomplished, the specimen is placed in a 3 per cent solution of sodium hydroxide or a 5 per cent solution of ammonium hydroxide. Gradual dissolution of the " humic " constituents occurs with concommitant clearing. Experience will indicate how long alkalination should be continued. Two to ten minutes is usually sufficient, but in some cases 8 hours is not excessive.

5. The specimen is then transferred, manually by forceps or lifter, to a shallow watch crystal, washed repeatedly with water and then covered with a 50 per cent solution of ethyl alcohol. After 5 minutes in the 50 per cent solution, the specimen is dehydrated gradually by successive changes of 70, 85, 90 and 95 per cent and absolute alcohol.

6. Following immersion in xylol for one minute, the specimen is placed on a microscope slide, wetted with a drop of xylol, covered with balsam or damar dissolved in xylol, and permanently sealed with a cover glass.

Most tissues investigated by this method show surprising mechanical strength and can be manipulated, with reasonable care, without difficulty. Fig. 5 is a macerated compression of *Neuropteris rarinervis*. In this specimen translucency was increased by a rinse in benzene between steps (5) and (6) described above. Many organic solvents may be used to remove selectively the impregnating bituminous compounds. A discussion of these solvents would be outside the scope of this paper, but mention of three — benzene, pyridine and aniline — will suggest the nature of their action. Successful recoveries have been made in somewhat less than 60 per cent of the attempts, but these reveal a wide range of structure : cuticle, epidermis, palisade and mesophyll parenchyma, xylem elements, ray parenchyma, sporangial cells, as well as the expected spores, pollens, etc.

A promising use for maceration may properly be considered dissection. I have used it for studying seeds in calcareous and pyritized coal balls. The specimen is immersed in a suitable acid bath in a shallow vessel.

With the aid of fine sharp needles the tissues may be peeled off, one by one, either in entirety or in large cell masses until the seed spore is recovered, nearly always complete and unbroken. Fig. 10 shows the seed spore of *Cordaicarpus florini* Darrah isolated by this method.

Inasmuch as some mechanical deformation and destruction result from the liberation of gas and the crumbling of attacked crystals, minimization of these effects is desirable. Some compensation can be gained by dispersing gum Arabic in the acid bath. Silicone oils serve the same purpose.

PEEL TRANSFER TECHNIQUES

Concurrently with refinement of maceration techniques there has been developed the so-called peel technique. The term " peel " is unfortunate because it has become associated with the primitive experiments which depended upon unstandardized cellulose acetates and nitrates which tended to embrittle and discolour with age. The basic technique was devised by Koopmans and improved by Walton whose inspiration came from an accidental observation by Nathorst (*ca.* 1908) that a dried film of collodion would peel off a little of the carbonaceous material from a compression. Within recent years the method has been highly perfected, particularly in the United States (Darrah, 1936).

Two distinct problems are involved in this technique : (1) selective maceration (" etching ") of the surface to be peeled and (2) compounding of a plastic colloidal mixture which upon setting will give an exact transfer of the structure of the fossil. Etching may be accomplished by various mineral acids though for silicified specimens hydrofluoric is necessary. Since the film cannot transfer

any more detail than is exposed by etching, the objective is to prepare the surface and follow the procedure with extreme care.

The peel solution, so-called, can be compounded from various constituents. There is no universal or ideal mixture, the purpose determines the most suitable. A mixture with a wide range of application may be prepared as follows :

115 gm. nitrocellulose (pyroxylon, grade 12-13 per cent nitrogen)
1,000 c.c. butyl acetate
200 c.c. amyl alcohol
10 c.c. toluol
5 c.c. dehydrated castor oil.

This mixture, which must be aged for approximately two weeks before use, will dry slowly without the inclusion of air bubbles. It may be thinned if desired by the addition of butyl acetate *without addition of the other* ingredients. Inasmuch as the solvent serves only as a vehicle for the nitrocellulose, a more dilute solution results in a thinner film. The highest purity pyroxylon is recommended. The film should have the best possible chemical and optical properties and be free from foreign inclusions. Ready-prepared commercial " dopes " are undependable. If these precautions are heeded, an excellent product will be obtained.

The procedure may be indicated briefly by the following schedule :

1. Grind and smooth (not polish) the surface to be studied.
2. Etch the surface with a suitable mineral acid (commonly hydrochloric or hydrofluoric).
3. Wash gently with running water, using care not to touch or agitate the etched surface.
4. Air dry the etched surface.
5. Cover, usually by pouring, the etched surface with nitrocellulose solution.
6. Allow film to dry at least 8 hours.
7. Peel film from matrix.

The resulting film, as noted above, is actually a thin transfer. Sections as thin as 0·5-1 micron have been measured. The thickness refers to the fossil structure, not the embedding nitrocellulose. The translucence of such preparations is far superior to that of conventional ground sections, a quality enhanced by the removal of soluble mineral and opaque insoluble clay particles. To obtain maximum permanence and optical clarity the film should be mounted in balsam or damar on a standard " non-corrosive " glass slide.

The possibilities of the peel transfer method are suggested by Figs. 7-9, 11-14. The degree of preservation is faithfully recovered, as the undamaged condition of the archegonium (FIG. 12) and young embryo (FIG. 13) will show.

It is not my intention to enumerate the discoveries made possible by refined techniques, but mention of a few examples, demonstrate the possibilities of these methods. The number of instances of compressions which reveal excellent cellular preservation is so great that they are no longer regarded as rare curiosities. Perhaps the most remarkable discovery of this kind was made by Harris (1939) who macerated Triassic sediments for specimens of the small liverwort *Naiadita lanceolata* (Bruckmann). This curious plant, known since 1844 by external form, revealed through Harris' preparations archegonia, perianth parts and gemma cups. Obviously, bryophytes, traditionally regarded as extremely delicate, afford opportunities for research as great as any other group.

Selaginellites amesiana (DARRAH, 1938) a compressed bituminized strobilus from Mazon Creek, Illinois, exhibited megaspores with contained gametophytes, some with cell nuclei and supposed nucleoli (FIG. 8). A few gametophytes had free nuclei and cell plates (which precede normal cell-wall formation). The preparations were nitrocellulose peels while the preservation was strictly bituminization.

Animal tissues as well as the more resistant plant structure may also be preserved by bituminization. We need only to recall the discovery of a mummified frog (from the Eocene brown coal of northern Germany) in which the skin contained expanded chromatophores (VOIGHT, 1935).

MICROTOME METHODS

Once the presence of recognizable, albeit collapsed, tissues was demonstrated, the compression type of preservation assumed new meaning. If tissues could be recovered by transfer or flotation subsequent to maceration, then they could be embedded and sectioned by established microtome technique. Experiment, indeed we may say practice, has proven this to be true. Two distinct embedding methods are practicable : (1) the celloidin method of Jeffrey (1916) originally devised for sectioning xyloid lignites and

other woody structures, and (2) a paraffin method devised by Halle (1933).

In either method, it is necessary first to demineralize the specimen completely because the presence of crystallites will damage the cutting edge of the microtome knife and tear the section. Foreign particles such as quartz or feldspar are particularly detrimental.

Unlike the methods discussed previously, the celloidin and paraffin embedding techniques involve rather costly supplementary equipment, however, such as is available in most biological laboratories. The celloidin method requires a sliding microtome of the Jung or Jeffrey-Thompson type while the paraffin procedure utilizes a rotary microtome. Free-hand sectioning, while possible, is extremely difficult because of the toughness of some materials and the friability of others. The materials are manipulated in accordance with standard laboratory practice with the exception of staining which, in most specimens, would be superfluous.

Microtome sectioning of fossil tissues has not gained general recognition, probably because of the prolonged and complicated proceeding involved in demineralizing and embedding. However, the remarkable preparations of Jeffrey, Halle, and others justify much wider application.

X-RAY PHOTOGRAPHY

The question naturally arises, in what condition do the plant tissues occur in the fossil specimen? Ground thin sections, of course, exhibit the undisturbed structure but because of inherent limitations cannot show thick or large features. During the summer of 1948 I commenced experiments with X-ray photography in an attempt to identify structure in certain calcified seeds removed from coal balls. The objective was to orient promising specimens so that in subsequent cutting and peeling, critical structures would not be destroyed or by-passed.

The gametophyte with the archegonium shown in Fig. 12 was oriented by this method prior to cutting (it should be noted, however, that the archegonium itself was not recognizable in the radiograph). Another radiograph (FIG. 15) of the base of the large embryo of *Lepidocarpon* (*Lepidocystis*) *glabrum* (DARRAH, 1949), shows the lobed construction with strong indication of the upturned arms, which appear to have a circular disposition. In this case the specimen had been sawn prior to X-ray photographing and it was known that the basal region was present. The radiograph confirmed the whole structure " in the round " which previously had been reconstructed from serial sections of three other specimens.

So far as I am aware there have been no previous published reports of the application of radiography to fossil plants, consequently a few of the problems encountered will be discussed briefly. A radiograph is simply a shadow picture recorded by a sensitized film. The object is photographed by X-rays, differences in density being recorded by corresponding differences in shadow effects.

The radiograph, being a shadow formation, is subject to distortion, particularly magnification which varies directly as the distance of the object from the sensitized film. The specimen shown in Fig. 15 measures 15·0 mm. in maximum width, the radiograph measures 15·4 mm. The distortion is, therefore, 0·4 mm. or approximately 2 per cent. The clarity or contrast of the image depends upon many factors of which two are critical: radiographic energy and focus. The amount of radiation which strikes the object may be expressed by the empirical formula:

$$\mathrm{Re} = \frac{\mathrm{Voltage}^{x} \times \mathrm{Current} \times \mathrm{Time}}{\mathrm{Distance}^{2}}$$

where, Voltage = kilovolts (kV)
Current = milliamperes (mA)
Time = seconds
Distance = inches or millimetres

The exponent x is a variable which for a given set of conditions may be taken as = 1.

The values most generally satisfactory for calcified and silicified specimens with thickness varying between 5 and 15 mm. were:

$$\mathrm{Re} = \frac{5\ \mathrm{kV} \times 5\ \mathrm{mA} \times 6\ \mathrm{sec.}}{12^{2}\ \mathrm{in.}} = 1{\cdot}00$$

Equipment capable of large focus was used for most of the investigation despite the fact that good radiographs were obtained with fine focus equipment of the type commonly used in dentistry. The example shown in Fig. 15 was taken with a large focus arrangement.

It is not necessary to enter into an elementary discussion of the techniques of radiology. The many applications of X-radiation and radiology in industry, medicine, and den-

tistry and the diversity of commercially available equipment have made such information readily accessible. An excellent introductory guide has been published by the Eastman-Kodak Company.

Various other techniques, chiefly microchemical, have been applied to the study of palaeobotanical materials to a slight extent. These experiments have been preliminary or purely exploratory. Elsewhere (DARRAH, 1941) I have suggested the promise which these hold.

SUMMARY AND CONCLUSIONS

Each of the methods described in this brief survey has contributed to a better understanding of the conditions of preservation and fossilization. Of greater significance, their combined effect has been demonstration of the fact that the original plant body, in varying degrees of degradation, is an integral part of many fossils, far more frequently than is commonly believed.

Some of the following conclusions are obvious, others are opinion, but they are stated boldly at the risk of pedantry.

1. The plant body is constructed of many chemical constituents which in varying degree resist subaerial oxidation and decomposition.

2. In embedded fossils, that is petrifactions in which the plant body is literally embedded in silica, calcite, or other mineral, this body may be recovered by dissolution of the matrix. The original tissues, somewhat degraded, remain and can be recovered subjected to microscopic examination and chemical analysis.

3. In bituminized fossils, i.e. 'so-called carbonized impressions, mummifications, compressions, etc., the plant tissues are impregnated with their own decomposition products. Many coals are composed principally of compressed plants which, though variously altered, are recoverable by maceration techniques.

4. Many organic compounds are virtually indestructible under conditions favourable for fossilization. A number of such compounds have been positively identified chemically.

5. Radiographs of compressions and embedded petrifactions show that in many specimens the tissues are neither crushed nor distorted.

6. With proper understanding of the nature of a given specimen, and the application of suitable technique, considerable knowledge hitherto unsought may be attained. Many specimens assumed to be worthless may contribute significant data towards the correlation of external form and internal structure.

7. There are no structures " too delicate for preservation ". Our methods have been inadequate, not our materials.

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THE ILLUSTRATIONS

The specimens selected to illustrate this brief summary suggest how in many cases it is possible to correlate external form with internal structure. Not only can a petrifaction be subjected to microtechnique, but a compression, in favourable instances, can be prepared to recover comparable data.

Maceration readily yields spores, pollens, cuticles, etc. Figs. 1, 2 and 10 show typical recoveries. The important factor is positive identification — known forms are subjected to maceration. With appropriate processing (strictly speaking, arrested or partial maceration) relatively large fragments of leaves or pinnules can be removed from associated rock matrix. The two specimens illustrated by Figs. 4 and 5 are compressions isolated by this method. The pinnule of *Neuropteris rarinervis* (Bunb.) is particularly interesting because it compares directly (FIG. 6) with a nitrocellulose peel transfer of a calcified pinnule preserved in a coal ball (FIG. 7). Considerable work has been devoted to this form which, incidentally, has been observed in attachment with *Myeloxylon* petioles.

High fidelity peel transfers can be obtained with both compressions and petrifactions. A single example of the former category is sufficient to indicate the thinness and fidelity attainable, the endosporal gametophyte of *Selaginellites amesiana* (Darrah) shown in Fig. 8. Peels of coal balls are innumerable. I have for instance made several thousands from specimens of *Lepidocarpon glabrum* (DARRAH, 1949) among them many gametophytes and embryos. A fine archegonium is shown in Fig. 12 and a recently discovered young embryo, the smallest yet observed, in Fig. 13. The large embryo in Fig. 14 has been described previously but is illustrated here for comparison with the radiograph shown in Fig. 15.

The completeness, with which *Neuropteris rarinervis* and *Lepidocarpon glabrum* are known, by benefit of diverse techniques, presages a rich return for our labours.

EXPLANATION OF PLATES

PLATE 1

1. Spores of *Ptychocarpus unitus* (Brongn.) macerated from a compression. Mazon Creek, Illinois. × 250.

2. Seed spore macerated from a compression of *Cordaicarpus fairchildi* White. Henry County, Missouri. × 5.

3. Pollen of *Cordaianthus shuleri* Darrah. Coal ball, Shuler Mine, Dallas County, Iowa. Nitrocellulose peel. Note that the pollen grains are entire and have been stripped by moderately deep etching in the surface. × 264.

4. *Sphenopteris* sp. Macerated and bleached compression. Carboniferous (Conemaugh): Fair Oaks, Allegheny County, Pennsylvania. × 2.

5. *Neuropteris rarinervis* (Bunb.). Macerated and bleached compression. Carboniferous: Henry County, Missouri. × 2.

6. Same specimen as No. 5. Pinnule enlarged to show venation and lacunar structure of mesophyll. × 22.

7. *Neuropteris rarinervis* (Bunb.). Nitrocellulose peel of a pinnule. Coal ball, Shuler Mine, Dallas County, Iowa. The specimen has been so ground that a longitudinal section was obtained. × 60.

8. *Selaginellites amesiana* (Darrah). Megaspore with gametophyte preserving nuclei and supposed nucleoli. Nitrocellulose peel, compression of a strobilus. Carboniferous : Mazon Creek, Illinois. × 200.

PLATE 2

9. *Cordaicarpus Florini* Darrah. Nitrocellulose peel, longitudinal section through apex of seed showing micropyle, summit of megaspore, and tissues of the pericarp. Coal ball, Shuler Mine, Dallas County, Iowa. × 60.

10. *Cordaicarpus Florini* Darrah. Seed spore macerated from a mechanically isolated seed. Coal ball, Shuler Mine, Dallas County, Iowa. × 22.

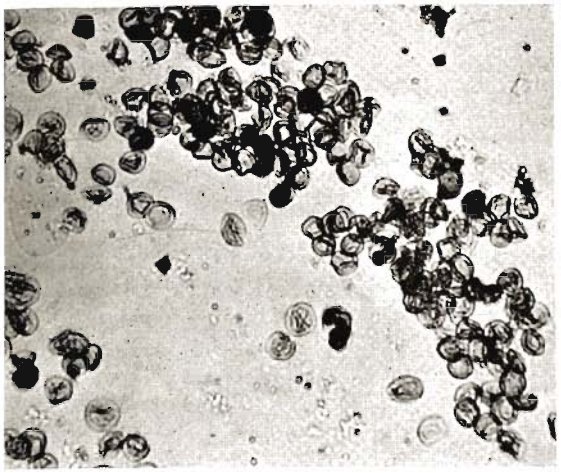
11. *Lepidocarpon glabrum* Darrah. Sporangium, bract, pedicel and abortive spores. Nitrocellulose peel. Carboniferous : Shuler Mine, Dallas County, Iowa. × 5.

12. *Lepidocarpon glabrum* Darrah. Nitrocellulose peel showing a portion of the gametophyte with an archegonium. Carboniferous : Shuler Mine, Dallas County, Iowa. × 60.

13. *Lepidocarpon glabrum* Darrah. Nitrocellulose peel showing young embryo Carboniferous : Shuler Mine, Dallas County, Iowa. × 60.

14. *Lepidocarpon glabrum* Darrah. Nitrocellulose peel. "Mature" embryo with lobed base. Vascularization is evident. Carboniferous : Shuler Mine, Dallas County, Iowa. × 5.

15. *Lepidocarpon glabrum* Darrah. Radiograph of the specimen shown in Fig. 14. Note the more dense area, the base, with strong indication of the lobes. Approximately natural size (see text for explanation of linear distortion).



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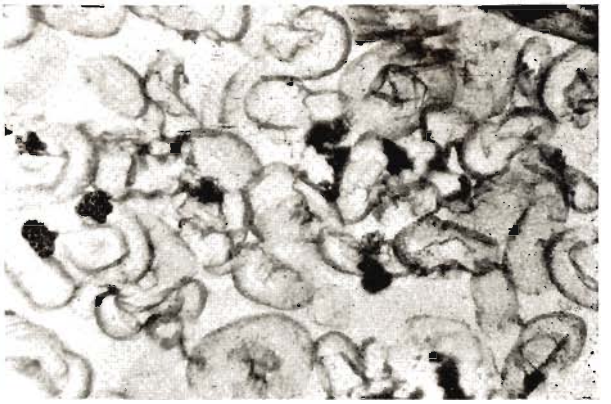

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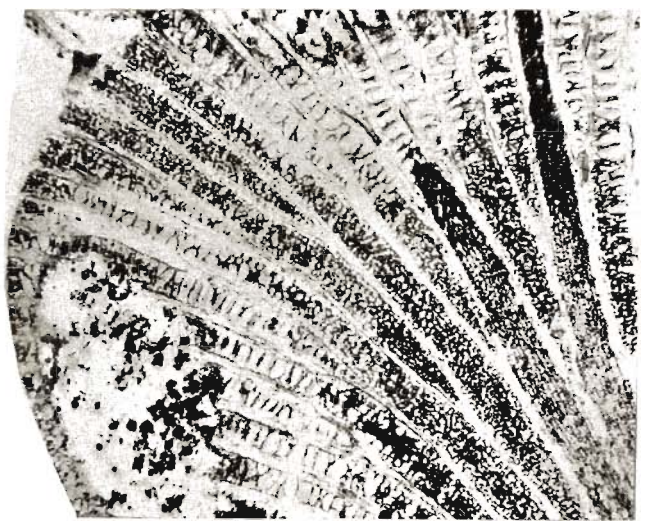

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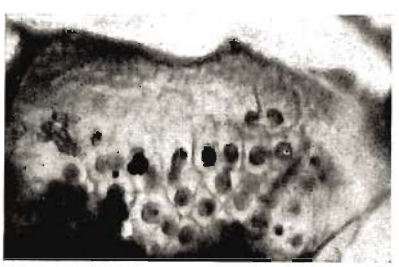

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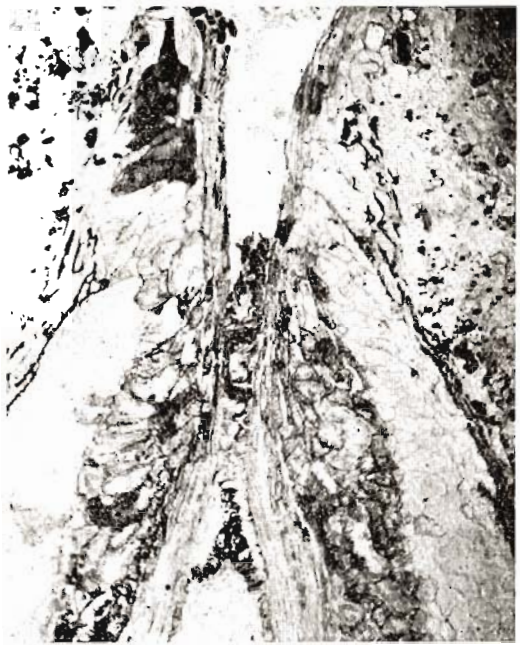

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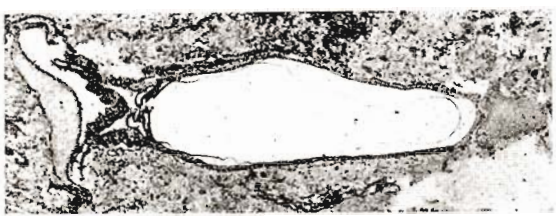

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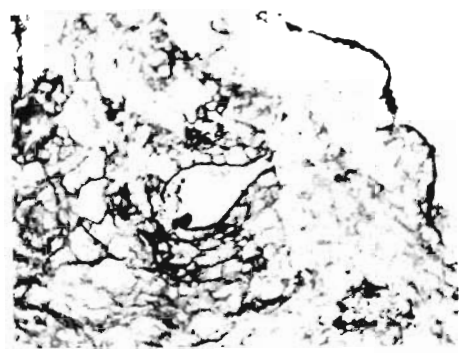

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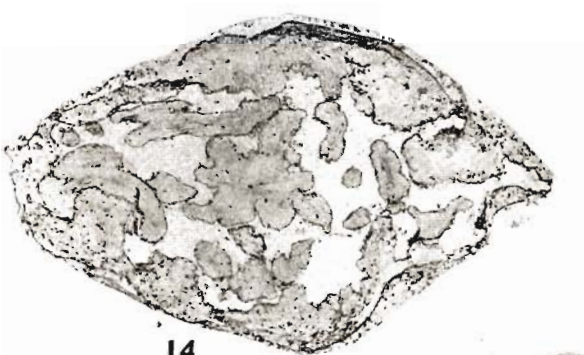

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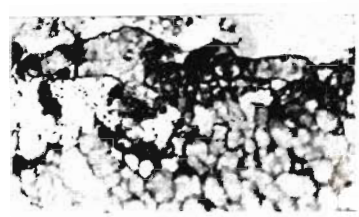

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