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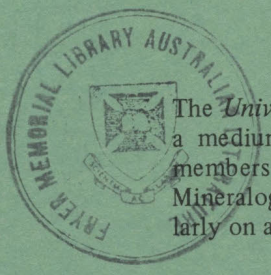

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VOLUME 11 NUMBER 1

Laboratory Techniques for Extraction of Palynomorphs from Sediments

D. PHIPPS and G. PLAYFORD

LABORATORY TECHNIQUES FOR EXTRACTION OF PALYNOMORPHS FROM SEDIMENTS

by **D. Phipps and G. Playford**

(With 1 text-figure and 3 plates)

ABSTRACT. A detailed description is given of physical and chemical procedures found to be effective in the recovery and concentration of palynological microfossils (specifically miospores and organic-walled microphytoplankton) from the normal lithological range of palyniferous sediments. It is emphasized that no simple 'rote' sequence of pre-determined steps is applicable, inasmuch as each potentially productive sample requires, to a greater or lesser extent, individual treatment if optimal results are to be achieved.

INTRODUCTION

During the twenty years that palynological teaching and research have been conducted in the University of Queensland's Department of Geology and Mineralogy, the procedures adopted for liberating palynomorphs from their host sediments have evolved from a fairly standardised routine, relying mainly on chemical reactions, to a more varied range of physical and chemical techniques. The changes have been brought about by a number of circumstances, including the publication of several excellent technical accounts (cited subsequently herein), verbal and written communications from colleagues located in other Australian and overseas laboratories, and, not least, by the innovative approaches of several of the Department's laboratory assistants of whom the first author (DP) is the most recent. The aim, as in all laboratories servicing palynological research, is to obtain from a given sediment a fully representative suite of its component palynomorphs, in as good a state of preservation as possible, free of as much extraneous material as possible, and hence appropriate for detailed microscopic examination and evaluation. The present account is, therefore, a stocktake of the laboratory procedures that we have found by experience to be most compatible with that aim, particularly with regard to the extraction of small spores and pollen grains (miospores; Plate 1, figs 1-6) and organic-walled microphytoplankton (acritarchs and dinoflagellate cysts; Plate 2, figs 1-6), mainly from pre-Quaternary sediments. For the recovery of megaspores we follow the procedures detailed by Dettmann (1965).

Many techniques and treatments have been experimented with in our laboratory. Some proved successful but were nonetheless discarded as standard procedures because they were not successful enough to compensate for the time involved. These methods are included in the paper as they may be useful for overcoming specific problems or may offer scope or challenge for further development.

Pap. Dep. Geol. Univ. Qd., **11** *(1)*: 1–23, Pls 1–3, October 1984

PREVIOUS LITERATURE

An exhaustive review of prior literature concerned specifically or incidentally with palynological preparatory techniques is beyond the scope of the present paper. However, we would draw attention to several publications that we have found to be particularly relevant and helpful. One of the most informative accounts is that of Gray (1965), who produced an especially comprehensive and well-explained survey of physical and chemical techniques based upon both Quaternary and older sediments and making extensive reference to earlier pertinent literature. Gray very properly stressed the need for an individualistic approach to sample processing, whereby due regard is given to lithological characteristics and to the desirability of successive monitoring of the residue in the course of the processing.

Extractive techniques utilized in laboratories of the Geological Survey of Canada were detailed in a very practical and explicit manner by Barss & Williams (1973). Their account also included a section dealing with the wholly mechanical processing of sediment for calcareous nannofossils (not considered here).

Among other papers that provide useful information, the following are noteworthy: Funkhouser & Evitt (1959), Staplin, Pocock, Jansonius & Oliphant (1960), Brown (1960), Balme & Hassell (1962), Kidson & Williams (1969), and Doher (1980).

CHEMICAL HAZARDS AND SAFEGUARDS

Many of the chemical reagents used in palynology processing are dangerous; moreover, hazardous products can be formed as byproducts during processing. Adequate ventilation of the laboratory and fume evacuation (via efficient fume cupboards) are basic prerequisites.

Chemical wastes should be poured down the sink with as much dilution as possible; thus it is advisable to have a tap running (preferably in fume cupboard) while wastes are being discarded.

Protective (but not awkwardly burdensome!) clothing and footwear should be worn. Whilst handling hydrofluoric acid (HF), strong acid-proof safety gloves must be worn and regularly inspected before use for possible flaws. Safety glasses are recommended when undertaking acetylation (Step 6; Text – fig. 1) as that process can be explosive.

The dangers of HF can scarcely be overemphasized (see, for example, Shewmake & Anderson 1979). The acid's fumes are lethal. Aqueous HF, if spilt on the skin, can produce excruciatingly painful and disfiguring effects: progressive tissue damage that may result in serious injury and disability. Prompt remedial action, as follows, is essential if a spillage occurs: (a) irrigate the affected area thoroughly; (b) apply calcium gluconate cream; (c) seek medical attention urgently if symptoms are severe, in which case injection of calcium gluconate may well prove necessary. Obviously, it is imperative that any procedure involving the use of HF be undertaken within a fume cupboard.

HF digestion of rocks produces fluosilicic acid, H_2SiF_6, from reaction with silicates (Step 4; Text-fig. 1); this is a toxic irritant. As some H_2SiF_6, and also unused HF, may be in the supernatant after centrifugation of the part-demineralised residue (Step 4), it is necessary to pour into a fume-cupboard sink with the tap running.

Zinc bromide ($ZnBr_2$) solution is extremely corrosive and toxic, and therefore requires careful handling to avoid spillage. If the latter occurs, the affected areas should be washed thoroughly. When pouring down the sink, the $ZnBr_2$ should be well diluted with tap water.

The gaseous nitrogen products formed during oxidation (Step 7) are toxic, so this step must be carried out in a fume cupboard.

As mentioned earlier, acetylation can be explosive if water comes into contact with the sulphuric acid/acetic anhydride mixture. It is advisable to perform this step in a fume cupboard with the front panel lowered as far as possible to act as a shield. Safety glasses should be worn.

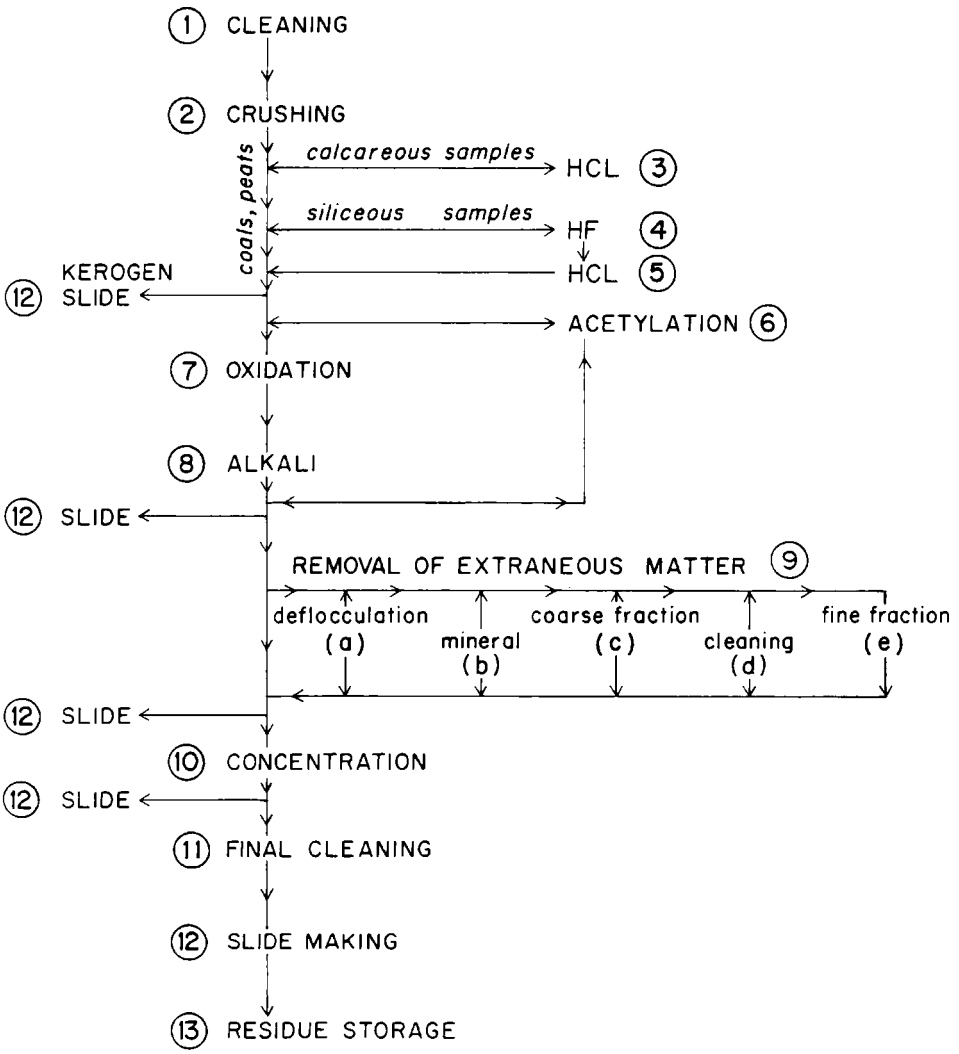
Some of the processing steps may produce inordinately violent reactions. *With acetylation as a crucial exception*, such reaction may be ameliorated by squirting with acetone or distilled water from squeeze bottles which should therefore be conveniently at hand.

EQUIPMENT AND GENERAL PROCEDURES

Care must be taken during processing to select the correct equipment and utensils, and to keep these and the samples free from introduced contamination and also from cross-contamination. Large (600 ml) pyrex beakers are preferable to use during removal of calcareous material (Step 3), as the reaction can be very strong and frothy. When using HF (Step 4), polypropylene beakers and centrifuge tubes and teflon stirring rods must be used. Either nickel or copper crucibles can be used for boiling samples in HF (Step 4). Some etching of the crucibles does occur; however, an insoluble fluoride forms on the crucible surface, thus providing it with some protection. A nickel crucible is preferable if introducing hydrochloric acid (HCl) into Step 4.

Some etching of glassware surfaces will occur during processing as some HF is formed in the chemical reactions involving the removal of fluorides in Step 5 and, to a lesser extent, during oxidation (Step 7). It is necessary to discard any glass centrifuge tubes that may be weakened through prolonged usage and likely to break during centrifuging.

The appropriate size of centrifuge tube should be selected for any particular processing stage. Either too much or too little residue in a tube risks the loss of palynomorphs or may render the processing difficult or ineffectual. Note that when the term 'centrifuge' is used in a verbal sense herein, it is intended to mean the application of centrifugation to produce a well-compacted residue which, on careful decantation, will be as deliquified as practicable. The requisite rate and duration of centrifugation depends in part on the particular centrifuge's arm radius and the tube capacity, and cannot therefore be given absolute values of r.p.m. and time that will apply to all



Text-fig. 1. Flow chart for palynological processing showing the sequence and interrelationships of the Steps 1-13 as detailed in text.

centrifuge models. In our laboratory, using an MSE 'Super Minor' centrifuge, we normally apply a rate of 2500 r.p.m. for 2 minutes to achieve residue compaction. However, when fluoride gels are abundantly present after hydrofluoric acid digestion (Step 4), the time may need to be extended to, say, 5 minutes. If organic particles are evident in the supernatant at any stage after centrifugation, it must be re-centrifuged for an increased time.

Successive steps in the processing schedule are monitored by taking a small sample of residue, via a pasteur pipette, and examining it under the microscope. This can be done by making a temporary 'wet' slide or by placing a drop or two on a small watch glass with a little water added. The wet slide has the advantage of being flat and small palynomorphs can be detected easily; however, the mounted material cannot readily be retrieved *in toto*. The material from a watch glass is readily retrievable; hence, frequent residue samples can be taken without loss. Further, watch glass material has the advantage of showing the overall 'state' of the residue; i.e., whether or not the palynomorphs are free, if 'fines' are floating on the surface, and large unwanted organic particles are sinking.

All equipment and utensils must be kept scrupulously clean to avoid contamination. Samples are normally processed in batches of four. All utensils are numbered and each sample assigned a corresponding number. Like-numbered samples and utensils (e.g., beakers, centrifuge tubes, pipettes) are thus exclusive to each other in the processing batch. Pasteur pipettes and screening materials are discarded after use.

Care must be exercised during preparation of chemical reagents so as not to introduce dust or fibre, and analytical reagents are used where possible. Wash-bottle jets must not come in contact with residues; otherwise contamination may result. Obviously, the laboratory must be kept clean and as free from dust intrusion as possible.

STOCK SOLUTIONS AND MOUNTING MEDIUM

Acids. Where an acid solution is specified as being, for instance, 10%, it is a one-tenth dilution from the concentrated form (i.e., 1 part acid : 9 parts distilled H_2O, by volume). Similarly, a 50% acid solution is a one-half dilution from the concentrated acid (1:1 by volume).

Acetylation mixture (Step 6). 1 part conc. sulphuric acid (H_2SO_4) : 9 parts acetic anhydride [$(CH_3CO)_2O$]. *Caution*: no water (see p.3).

Schulze solution (Step 7). 30 ml saturated potassium chlorate ($KClO_3$) : 90 ml conc. nitric acid (HNO_3).

Potassium hydroxide (KOH) solution (Step 8). 5% solution: dissolve 5 g of KOH crystals in 100 ml of distilled H_2O.

Zinc bromide ($ZnBr_2$) solution, S.G. 2.0 (Steps 9 and 10). Dissolve 500 g of $ZnBr_2$ crystals in 220 ml of 10% HCl, warming on hot plate if necessary. Filter through glass filter paper (the solution will dissolve ordinary filter paper and nylon screening material). Check S.G. and adjust if necessary.

The following dilutions apply for S.G. reductions (from 2.0):

Requisite S.G.	H_2O	:	$ZnBr_2$ (S.G. 2.0)
1.2	4	:	1
1.25	3	:	1
1.33	2	:	1
1.5	1	:	1
1.6	1	:	1.5
1.67	1	:	2
1.75	1	:	3
1.8	1	:	4

Glycerine jelly mountant (Step 12). Ingredients: 10 g gelatine powder, 60 ml glycerine, 0.25 g copper sulphate, 60 ml distilled H_2O. Add gelatine to warm H_2O and heat in a water bath until dissolved. Add glycerine and copper sulphate. Stir gently (to avoid bubbles) until well dispersed (*ca* 10 minutes). Filter through 25 μm screening material. If stained mountant is required, add a few grains of saffranin to melted standard jelly.

Thiomersal (merthiolate) solution (bacterial and fungal inhibitor). Prepare stock solution of 1 : 1000 (i.e., 1 g/litre), and use 1 part stock in 100 parts glycerine jelly (maximum use is 1 part in 10).

Copper sulphate ($CuSO_4$) solution (fungal inhibitor). Prepare 3% stock solution (i.e., 3 g/100 ml).

Brij 35 (polyoxyethylene lauryl ether; a non-ionic detergent). One-third of a teaspoon is dissolved in 500 ml warm distilled H_2O. Filter through 0.45 μm millipore filter.

PROCESSING PROCEDURES

Described below is a series of 13 laboratory processing 'steps'. These do not necessarily constitute a rigidly sequential schedule insofar as some steps may be omitted according to the sample lithology or perhaps its weathered nature, or to the condition/composition of the residue as revealed by test slides during the course of processing. For instance, Steps 3-5 would normally be omitted in the processing of coals, and Step 7 from naturally oxidized material. Text-fig. 1 depicts the normal sequence of processing steps and their interrelationships. Plate 3 illustrates two end-products, from strew slides of final palynological residues.

STEP 1: CLEANING

The objective here is to ensure that samples supplied (outcrop, core, sidewall core, cuttings) are clean and free from surface contamination before actual processing. Outcrop and core samples are washed under a running tap, and, if sufficiently coherent, are scrubbed with a stiff, clean brush. From the surface of sidewall cores, scrape off surficial drilling mud with a clean scalpel and wash under running tap. Samples of cuttings require special initial handling, because of inherent drillhole contamination. Normally, such samples should be placed

in a large beaker and well-covered with boiling water; decant and repeat with cold water until supernatant is clear.

The cleaned sample material is left to dry by placing between sheets of paper towel. Record the processing number on the towelling.

STEP 2: CRUSHING

The amount of sample to be processed for appropriate palynological yield depends on the relative proportions of organic and inorganic matter contained in the sample. With experience, a technician can usually estimate quite realistically the appropriate processing amounts for the various lithologies (though, of course, where small quantities only are supplied there is little choice). Barss & Williams (1973, p. 4) suggested the following amounts, which provide a reasonable guide for the uninitiated: 5-10 g (carbonaceous shales-siltstones, coals); 25-30 g (calcareous or siliceous shales-siltstones, argillaceous limestones, argillaceous sandstones); and 35-50 g (sandstones, limestones).

For physical disaggregation, place the cleansed and dried sample between two aluminium pie dishes and crush with a clean hammer to *ca* 1-2 mm fragments. Use several thicknesses of pie dishes if necessary. Discard dishes after use. N.B. A mortar and pestle are not recommended for crushing as their surfaces are too porous or prone to pitting and are thus liable to act as media for contamination.

STEP 3: PRE-HF TREATMENT

Carbonates

Calcium and magnesium carbonates must be removed from the sample prior to HF digestion (Step 4). Otherwise, fine insoluble precipitates of secondary fluorides (CaF_2, MgF_2) will form. Small amounts of these will be removed in HNO_3 during oxidation (Step 7), but large amounts are very difficult to eradicate. Carbonates are thus normally solubilized with HCl after physical disaggregation. However, HCl can cause problems if the sample contains a high proportion of clays.

Hydrochloric acid. Several dilutions of HCl are used according to the nature (carbonate content) of individual samples. Mindful of the possible corrosive effects of HCl on the palynomorphs, it is desirable not to exceed the amount/strength of the acid that is requisite for dissolution of the carbonate. Accordingly, the crushed dry sample is first tested (in a large beaker) for carbonate reaction with a small amount of 10% HCl. If this initial reaction is slight, small amounts of 10% HCl are successively added until acceleration of the reaction ceases. If the initial reaction (with 10% HCl) is strong, 50% HCl is added in successive small quantities until the reaction ceases to accelerate. Because dolomite ($CaCO_3.MgCO_3$) is soluble in hot HCl, dolomitic samples should be heated in HCl on a hot plate using the same procedure.

Settling time for the decarbonated samples, now in a weak HCl solution, varies according to the amount of salts or clays in the supernatant, but at least one hour is recommended. If supernatant is clear, carefully decant liquid from

settled sample. Test the latter with fresh HCl to determine whether further acid treatment is required. It may be necessary to repeat the HCl treatment if the sample has a high carbonate content. The sample must then be washed free of all soluble calcium and magnesium: at least three washes with distilled H_2O, either by decanting or by centrifuging. Clays can be partially decomposed by the action of HCl, which causes a dispersion of colloidal matter in the supernatant. Small palynomorphs could become entangled in these colloids and unwittingly poured away during decantation. Therefore it is safer to centrifuge when processing clayey samples.

Orthophosphoric acid (H_3PO_4). Carbonates can be removed with H_3PO_4, which is possibly more efficient than HCl in eliminating dolomite. With a 25% solution, the reaction tends to be slow, and a thick white precipitate sometimes forms; this increases the density of the supernatant and requires several washes to remove. Samples treated with H_3PO_4 digest quickly in HF (Step 4) with a reduction in fluoride precipitants formed. Use of H_3PO_4 appears to be favourable where dolomite is present; however, slowness of reaction, increased density of supernatant, and the need for repeated washings militate against its use as a standard method for dolomite removal.

Clays

The complex lattice structure of clays locks in several different cations. When the clay structure is broken, partially by the action of HCl in this step and later with HF in Step 4, these cations are freed to form highly insoluble fluorides that require several treatments with hot HCl to remove (Step 5). In an effort to remove as much clay mineral as possible prior to HF treatment, the following chemicals have been experimented with: E.D.T.A., Calgon, and sodium silicate.

E.D.T.A. (ethylenediaminetetraacetic acid-disodium salt). This is a chelating compound which removes free calcium, magnesium, and other cations from solution. One sample, treated with a 10% solution, showed a reduction in fluorides subsequently formed, but this result could not consistently be repeated with a number of other samples. The efficacy of this procedure probably depends on the quantities of *free* cations in solution and thus is too selective to be of standard application.

Calgon (sodium hexametaphosphate). Calgon is commonly used as a dispersal agent for clays, and it removes calcium and magnesium from solution. Care must be taken in using Calgon as some contaminants (e.g. diatoms) were suspected to have come from the commercial product. It would be preferable therefore to obtain the analytical grade of this chemical. A 2% solution was utilized and similar results were obtained as for E.D.T.A.; i.e., consistently satisfactory results could not be achieved.

Sodium silicate (Na_2SiO_3). Adding Na_2SiO_3 solution (20%) to clays does appear to affect the clay structure by making it more readily digestible in HF (Step 4). The supernatant of clay treated with Na_2SiO_3 contained a higher proportion of aluminium than untreated clay. In some samples, the addition of

Na_2SiO_3 increased the viscosity of the solution, so care must be exercised during decantation.

Detailed reference to the reactions of Na_2SiO_3 is difficult to find. Gray (1965, p. 581) referred to Na_2SiO_3 as being a 'silica depressant'. According to Van Nostrand's Scientific Encyclopaedia (1968, p. 1644), Na_2SiO_3 when mixed with atmospheric CO_2, or with sodium carbonate or ammonium chloride solutions, produces silicic acid as a gelatinous precipitate. The latter may account for increased viscosity experienced with some clays. Mellor (1925, p. 491) recorded that when kaolinite is mixed with Na_2SiO_3, analcite is formed.

Standard procedures adopted by this laboratory are to routinely use HCl with heat, then wash four times, with careful decanting after each wash. Even a sample that does not appear to be calcareous often reacts mildly with HCl when heated. It also appears to help disaggregate the sample further, rendering HF treatment more effective. The other methods outlined above have certain advantages that should be kept in mind for specific problems.

Summary of Step 3:

(a) Add successive small amounts of 10% or 50% HCl whilst stirring.
(b) Heat to approximately 50°C.
(c) Allow to settle (for one hour at least).
(d) Wash four times with distilled H_2O, decanting carefully after each wash. (Two washes are normally sufficient if sample is only slightly calcareous.)
(e) Transfer residue to polypropylene centrifuge tube. Centrifuge.

STEP 4: SILICATE REMOVAL

HF is used, with great care (see pp. 2–3), to remove silica and silicates. As these comprise the bulk of sample matrices (with the exception of carbonates and coals), the action of HF causes digestive disaggregation of the sample with resultant release of the organic material. Even dolostones and limestones that appear to have disaggregated in HCl (Step 3) should subsequently be treated with HF to remove any component silica, silicates, or metal oxides.

The complex reactive nature of HF and the unknown inorganic chemical composition of the sample material make it difficult to state conclusively which is the most effective acid strength and method of usage to adopt.

HF is a weak acid in that it dissociates only partly into H^+ and F^- ions; viz., $HF \rightleftharpoons H^+ + F^-$. In HF, silica and silicates are converted to silicon tetrafluoride (SiF_4) and fluosilicic acid (H_2SiF_6). Metal oxides are converted to fluorides.

Cold conc. hydrofluoric acid. Using 600 ml polypropylene beakers and teflon stirring rods, carefully add approximately 300 ml of conc. HF to sample. As the reaction can be violent, a wash bottle of water should be at hand to slow the reaction if necessary. The commonly used technical grade of conc. HF (actually *ca* 70%) has a specific gravity of 1.25. This is increased if fluosilicic acid, fluo-

rides, or silicic acid are present; because any or all of these may have been produced as reaction byproducts and some organic matter may be held in suspension, great care must be taken in decanting (i.e., after the cold digestion of some 24 hours' duration). It would be preferable to transfer the contents of the beaker to polypropylene tubes and centrifuge; however, as HF vapours are so hazardous, sealed units would have to be employed to prevent atomising the vapours and releasing them into the laboratory. Wash/centrifuge the residue three times with distilled H_2O. The organic material from cold HF digestion has been found in our laboratory not to oxidize and release its palynomorphs as freely as do samples boiled in HF, thus suggesting that hot HF does affect the organic fraction.

Hot hydrofluoric acid. Copper or nickel crucibles and teflon stirring rods are used when boiling samples in HF. Copper crucibles probably disperse heat more efficiently; however, nickel crucibles last longer if HCl is present during digestion. A sand tray on the hot plate prevents 'hot spots' by providing even heat dispersal, and also provides some safety if spillages occur.

Several dilutions have been used in the laboratory. As with the HCl treatment, 'tailor making' the dilution as appropriate for a particular sample proved advantageous and also is safer, as most of the HF will be used in the reaction.

The sample is washed from the polypropylene tube (from Step 3) with distilled H_2O into a crucible. Small amounts of conc. HF are added whilst stirring; this is continued until there is no visible reaction. Boiling on the hot plate, with occasional stirring, for 30 minutes usually suffices for thorough disaggregation and silicate dissolution. Care must be taken not to allow the residue to boil dry or to 'spit' into adjacent crucibles of the processing batch.

A fine white precipitate sometimes forms on the surface; this could be fluosilicic acid (H_2SiF_6) or undigested clays. Add a small amount of conc. HF to dissolve clays. If there is no effect, add hot distilled H_2O and boil for a few more minutes (H_2SiF_6 is soluble in hot water). If a thick gel forms, this could be silicic acid (H_2SiO_3) or fluorides. Add HF, which dissolves silicic acid and *some* fluorides, but care must now be exercised with centrifuging. Add only sufficient HF to break down the gel, then add hot distilled H_2O and centrifuge, progressively adding contents of crucible to the centrifuge tube with each centrifugation, if necessary. If the addition of HF has no effect, the gel will be eliminated as per Step 5.

Dolostones and other calcareous samples that release calcium and magnesium as their matrix is dissolved are best treated with a combination of HCl and HF. This laboratory utilizes the following procedure with no apparent adverse effect on palynomorphs. The residue is washed into the crucible with 10% HCl. Small quantities of conc. HF are added until visible reaction ceases and then boiled. If the sample does not disaggregate and lumps gather on the bottom of the crucible, add HCl and boil and stir until the material disaggregates.

As fluorides and silicic acid are more soluble in hot water, wash/centrifuge the residue three times in hot distilled H_2O.

Summary of Step 4:

(a) Wash contents of centrifuge tube into crucible with either distilled H_2O or 10% HCl.
(b) Add successive small amounts of HF while stirring.
(c) Boil until disaggregated.
(d) Transfer to polypropylene tubes and centrifuge.
(e) Wash/centrifuge three times with hot distilled H_2O.

STEP 5: FLUORIDE REMOVAL

Fluoride precipitates, resulting from Step 4, must be removed to ensure release of the organic matter. Fluoride is observable in the residue as either a thick gel or a fine crystalline precipitate. Because solubility of fluorides increases with temperature, keeping the residue warm to hot from Step 4 hastens this step. Other aids for fluoride removal include use of a mechanical agitator to break down the gel whilst in the centrifuge tube; and, to ensure effective contact between solvent and solute, using 250 ml pyrex beakers with constant stirring.

Fluorides are salts of a weak acid; i.e., HF dissociates only slightly in solution. When a strong acid is added to fluorides they are removed from solution; e.g., $M^+ + F^- + H^+ \rightleftharpoons HF + M^+$. Thus, the fluoride is removed from solution as HF when H^+ is added. Heating the solution further increases the dissociation of MF as some HF will volatilize causing more fluoride to dissociate to form HF.

The acid most commonly employed to remove fluorides is HCl. Some fluorides (e.g., CaF_2, MgF_2) are more soluble in HNO_3; however, as this acid oxidizes organic matter, it is unwise to use at this stage of processing.

Sulphuric acid (H_2SO_4) is unsatisfactory as it produces insoluble sulphates which then have to be removed.

A saturated solution of boric acid (H_2BO_3) will successfully eradicate fluorides. However, it is a slow reaction and requires the residue to be stood in a large volume of the acid for at least 24 hours.

This laboratory has experimented with various dilutions of HCl. Conc. HCl does not appear to break down fluorides as efficiently as the diluted acid. 10% HCl was found to be not strong enough. The dilution which gave the most consistently satisfactory results was 50%.

The method usually adopted is to add a small amount of hot distilled H_2O to the residue in tubes from Step 4 and to proceed as follows. Agitate residue on mechanical mixer and pour into 250 ml beaker. Add more hot distilled H_2O to 125 ml mark, then add conc. HCl to beaker capacity. Place on sand bath on hot plate and bring to boil, stirring frequently. Remove from heat. Progressively add contents of beaker to polypropylene tube and centrifuge. Use caps on the tubes as toxic gases are present. Repeat HCl treatment if necessary until the residue material appears to be free from any coagulation. Some fine crystalline precipitate may be evident; this can be removed during Step 9(b)

(heavy mineral separation).

As the quantity of residue is now reduced (by fluoride removal), it is important to choose an appropriate (normally reduced) tube size (see also p. 3). Wash/centrifuge the residue three times with warm distilled H_2O. Gases may still be present, so do not vigorously shake the tube; add a small quantity of distilled H_2O to the tube and agitate on the mixer, fill the tube with further H_2O and carefully invert to mix.

Summary of Step 5:

(a) Add a small amount of hot distilled H_2O to residue in centrifuge tube and agitate to mix. Wash residue into 250 ml beaker with hot distilled H_2O. Fill to 125 ml mark.
(b) Add conc. HCl to beaker capacity.
(c) Heat to boiling point on sand bath on hot plate, stirring frequently.
(d) Transfer to polypropylene tube. Centrifuge.
(e) Repeat (a) – (d) if necessary.
(f) Wash/centrifuge three times with warm distilled H_2O.

STEP 6: ACETYLATION

Following fluoride removal, test slides are taken of the residue to assess the organic material for oxidation. If the material contains palynomorphs enmeshed in large amounts of organic matter, acetylation may be an appropriate procedure for their release: either in lieu of oxidation (Step 7) or in combination with it (before or after). We have not adopted acetylation as a standard processing technique, but it is particularly effective for Quaternary-age sediments containing a high proportion of relatively undecomposed, cellulosic plant material.

Acetylation breaks down cellulose to form an ester that is soluble in glacial acetic acid (CH_3COOH): see Gray (1965, pp. 544–545, 553–555). *Caution:* great care must be exercised during the acetylation procedure as the mixture can be explosive if water is present. All utensils must be scrupulously dry and kept away from any water. The procedure should be conducted in a fume cupboard and the technician should be protected by a facial shield.

The acetylation mixture is proportioned thus: 1 part conc. H_2SO_4: 9 parts $(CH_3CO)_2O$ (acetic anhydride). The mixture should be made freshly (i.e., just prior to use) and carefully (the reaction is exothermic).

Summary of Step 6:

(a) Wash/centrifuge residue three times in glacial CH_3COOH.
(b) Using glacial CH_3COOH, transfer residue to small (50 ml) beaker.
(c) Dry beaker contents on hot plate (low heat).
(d) Slowly add acetylation mixture (normally 10–20 ml) to dried residue and stir.

(e) Transfer to centrifuge tube, and place tube in water bath which is brought slowly to boil. When water bath is boiling, stop the heating and allow the tube to stand in the hot water for 15 minutes.
(f) Centrifuge and pour supernatant slowly down sink in fume cupboard, together with a large volume of water.
(g) Wash/centrifuge the residue three times in glacial CH_3COOH.
(h) Wash/centrifuge residue three times in distilled H_2O.

A wash with weak alkali can help clear the residue further of unwanted organic matter.

STEP 7: OXIDATION

Oxidation is performed in order to transform at least partially decomposed organic debris into alkali-soluble 'humic acids', thereby deleting unwanted organic matter and producing ultimately (with Step 8 *et seq.*) a palynomorph-rich residue. It is a delicate and crucial step inasmuch as the palynomorphs are themselves oxidizable and will be eliminated, along with the organic debris, if the oxidative process is not judiciously assessed beforehand and then monitored as oxidation proceeds. The method chosen should be as mild as possible, consistent with the aim of eliminating the unwanted organic fraction as far as possible but not to the detriment of any components of the palynomorph fraction. The latter will include forms of varying morphology and concomitant susceptibility to the oxidative treatment.

Consequently, it is most important to select carefully the appropriate oxidant and its strength, and to have some idea of the likely time to be allowed for the reaction. Astuteness and success, of course, come ultimately with experience! As a guide, the following should be noted in a pre-oxidation microscopic examination of the residue: amount and condition of (non-palynological) organic detritus and whether the latter enmeshes the palynomorphs; physical appearance of any visible palynomorphs (i.e., opacity or translucency, colour, preservational quality); and presence of HCl-insoluble fluorides. These factors will assist in deciding the oxidation method that might appropriately be employed for a particular sample. For instance, if the palynomorphs are light in colour and are associated with an unobtrusive amount of organic debris, a very mild oxidation (or even none whatsoever) would be indicated. If the palynomorphs are dark-coloured and accompanied by much unwanted organic material, an appreciably stronger oxidation would be applicable. As noted by Barss & Williams (1973, p. 8), it is preferable to underoxidize rather than overoxidize, because oxidation is an irreversible process.

Among the many oxidants that can be used (see, for example, Gray 1965, pp. 546–547), the two most favoured in palynological processing are Schulze solution and HNO_3.

Schulze solution (30 ml saturated $KClO_3$ solution : 90 ml conc. HNO_3) can be utilized in that proportion or in dilutions. For instance, successful gentle oxidation can be achieved with a 30% dilution and the application of heat (from water bath). This laboratory routinely uses HNO_3, mostly in its concen-

trated form. The more frequent use of it has possibly led to a more critical appraisal of the reaction, however, in experiments carried out with divided residues after Step 5, conc. HNO_3 consistently yielded better results than Schulze solution. 'Clumping' does not seem to occur as frequently in residues treated with HNO_3; this may well be due to the removal of HCl-insoluble fluorides.

When oxidizing with HNO_3, a violent reaction can occur if pyrite is present in the residue. For this reason, the reaction is best carried out in a small beaker rather than in a centrifuge tube. The reaction can be subdued if necessary by squirting with distilled H_2O from a wash bottle, or by standing the beaker in cold water. *Caution:* toxic nitrogenous gases are given off during oxidation, which should be carried out in a fume cardboard. Constant swirling of the beaker and the application of heat will ensure completion of oxidation; this is usually indicated by a change in colour of the residue from black/brown to dark brown and by the supernatant assuming a golden colouration. Average reaction time with conc. HNO_3 is 10 minutes.

Summary of Step 7:

(a) Cautiously adding successive small quantities of conc. HNO_3, transfer centrifuged residue (ex Step 5) to a small beaker.
(b) Allow any pyrite reaction to subside.
(c) Gently heat, with constant swirling of the beaker, on sand bath (on hot plate).
(d) Remove from heat when reaction begins.
(e) Allow this reaction to subside and re-warm if necessary.
(f) Add distilled H_2O and transfer to centrifuge tube (preferably glass).
(g) Centrifuge.
(h) Wash/centrifuge three times with distilled H_2O.

STEP 8: ALKALI TREATMENT

By treatment with a basic solution, as described below, the residue is palynologically concentrated further through dissolution of unwanted, oxidized organic matter (humic compounds). Again, this is a delicate operation because the hydroxide solutions conventionally used may dissolve or corrode at least some components of the wanted microfossil fraction; i.e., if the residue has been overoxidized in Step 7 and/or if the alkaline solution is of undue strength. It is prudent, therefore, to treat the residue initially with very weak alkali and observe the effect. Step 8 normally has a markedly propitious effect on the residue by ridding it of much of the non-palynomorph organic material with concomitant release and cleansing of the palynomorphs. If such does not occur with the initial alkali treatment, subsequent application of a more strongly basic solution, moderate heating, or even a short treatment in an ultrasonic tank can prove advantageous. The addition of a few drops of a nonionic detergent such as 'Brij 35' helps to inhibit 'clumping' effects.

If large amounts of organic matter are present, this step is best undertaken in a beaker as the solution can become very viscous and dark-coloured. After treatment, progressively add small amounts to a centrifuge tube with distilled H_2O, and carefully pour off the supernatant after centrifugation. Repeat washing/centrifugation with distilled H_2O until supernatant remains clear.

The following alkaline solutions may be used: 5% potassium hydroxide (KOH) or sodium hydroxide (NaOH); 10% ammonium hydroxide (NH_4OH), which because of its volatility should be made as stock solution at frequent intervals; 5% sodium carbonate (Na_2CO_3), which because it produces NaOH in solution has possibly little advantage over the latter; 10% or 20% sodium silicate (Na_2SiO_3), which like Na_2CO_3, produces NaOH in solution and moreover has the disadvantage of being an ideal medium for crystal growth.

We have found KOH solution to be the most satisfactory humic-clearing reagent, although we frequently use the less strongly reacting NH_4OH when there is any likelihood of otherwise eliminating thin-walled or delicately constructed palynomorphs.

Summary of Step 8:

(a) Half-fill centrifuge tube with distilled H_2O; agitate to mix.
(b) Add a couple of drops of 'Brij 35' stock solution, and approximately 1–2 ml of 5% KOH.
(c) Place cap on tube and invert several times to mix. Fill tube with distilled H_2O.
(d) Centrifuge and carefully pour off supernatant.
(e) If there has been any obvious reaction, wash at least three times with distilled H_2O. If not (and confirmed by test slide microscopic examination): (i) increase alkali strength; (ii) heat residue in water bath (but do not boil); and (iii) place tube in ultrasonic tank for up to one minute.
(f) Repeat above if necessary.

STEP 9: REMOVAL OF EXTRANEOUS MATERIAL FROM RESIDUE

Following the alkali treatment (Step 8), the residue is examined under the microscope, and some or all of the following procedures may be adopted before moving on to any final cleanup and concentration measures (Steps 10 and 11) that may be required. Step 9 aims to remove as much still-remaining unwanted matter as possible, and to ensure that the palynomorph residue is in an uncoagulated, free-moving state. This entails elimination of: (a) 'clumping' (flocculation) in residue; (b) mineral matter; (c) large organic fragments; (d) debris attached to palynomorphs and any excessively dark colouration of the latter; and (e) fine non-palynomorph material.

(a) The residue may exhibit a tendency to 'clump', whereby palynomorphs become attached to each other and to other organic particles. Often a wash at this point with HCl will resolve this problem. Fill the tube with 50% HCl, agitate, and heat (without boiling) in water bath for approximately 30

minutes. Centrifuge and discard supernatant. Add a few drops of 'Brij 35' solution to tube and wash the residue at least three times until supernatant remains clear. 'Brij 35' is an ether and is unaffected by the presence of acids or alkalis; as a nonionic detergent, it lessens the electrostatic clustering tendency of microscopic particles.

(b) Mineral particles can be removed from the residue by heavy-liquid separation, using zinc bromide ($ZnBr_2$) solution (S.G. 2.0) as the separative medium (*Caution:* see p. 3). It is essential for the residue to be non-coagulating; otherwise it may form a thick plug in the $ZnBr_2$ solution and prevent efficient separation of mineral matter from the organic matter. The residue should be slightly acidic to inhibit precipitation of $Zn(OH)_2$; this can be done by adding a few drops of 10% HCl to the residue and centrifuging/decanting before adding the $ZnBr_2$.

Depending on quantity of residue available, this procedure can be performed in a 15 ml or 50 ml centrifuge tube or in a U-tube of flexible, transparent plastic tubing (diameter *ca* 12 mm). In the latter case, use sufficient length of tubing to fit within a 100 ml centrifuge tube with approximately 2 cm of each arm protruding; lubricating the outside of the U-tube with glycerine will facilitate its unjolted removal after centrifugation. The floating organic fraction ('floats') can then be washed from the top by pinching the arms of the plastic tube with pliers just below the 'floats'.

If glass centrifuge tubes are used, the 'floats' can readily be extracted with a pasteur pipette, care being taken to gather any material adhering to the tube wall.

Having selected the appropriate centrifuge tube to be used, remove as much liquid as possible from the residue by centrifuging. Add the $ZnBr_2$ solution to half-fill the tube, mix thoroughly, and centrifuge at 1000 r.p.m. for about two minutes. Using higher speeds and longer centrifuging time may cause a coherent plug of the float fraction to form; this is difficult to remove cleanly as it will be disturbed during pipetting. Examine both 'floats' and 'sinks' under the microscope. If a clean separation has not resulted and palynomorphs are present in the 'sinks', the procedure should be repeated by adding $ZnBr_2$ to the 'sinks' and then adding the resultant 'floats' to the primary float fraction.

Once a satisfactory separation has been achieved, the organic matter is concentrated by transferring a small portion of the organic ('float') fraction to a centrifuge tube. Fill tube with distilled H_2O, centrifuge, and pour off supernatant. Progressively add the remaining 'floats' to the tube, centrifuging/washing between each addition, until all the concentrated organic residue has been compounded as the new residue, which is then washed and centrifuged three times, i.e. to remove all $ZnBr_2$.

(c) Large organic particles can be removed by sieving, using nylon screening material having mesh size of 170 μm or 200 μm. Care should be taken to ensure absolute cleanliness of the screening material before use, by thorough washing in distilled H_2O in an ultrasonic tank. Sterilization of the screen with ultraviolet radiation may also be desirable (but not with heat as the nylon is, of course, heat-susceptible). A small square (*ca* 8 cm x 8 cm) of clean screening

material is placed inside a small filter funnel and the residue is pipetted through this. Microscopically examine the material retained on screen; if no palynomorphs have been collected on it, discard the sieve and the extraneous matter. If any large palynomorphs have been collected, either make a separate slide or, if unaccompanied by obtrusive amounts of large organic particles, return them to the residue that has passed through the screen.

(d) If the walls of the palynomorphs are very dark coloured and/or have obtrusive debris adherent to their surfaces, half-fill the tube with distilled H_2O, and add a few drops of 'Brij 35' solution and of 5% KOH; mix by agitation and heat gently in water bath. Remove the tube frequently from the water bath and gently agitate contents. Centrifuge and discard supernatant. Examine the palynomorphs and if they are still not clean, half-fill the tube with distilled H_2O and place in ultrasonic tank for about 2 minutes; wash/centrifuge three times.

(e) 'Short-time' or differential centrifugation (see Gray 1965, pp. 571–572; Barss & Williams 1973, pp. 8–9) can be effective in removing fine organic debris. It is, of course, applicable only if the unwanted debris is of appreciably finer grade and lesser density than the microfossils, in which case the suspended 'fines' are decantable after the centrifugation. The selection of the appropriate centrifuge rate and the duration of the centrifuging depend on a number of factors (including composition and quantity of the residue, size of centrifuge tube, and radius of centrifuge arms) and is therefore a matter of practice and experience in a given laboratory situation.

Our procedure is to add a few drops of 'Brij 35' to the residue, mix thoroughly in the tube filled with distilled H_2O, and then 'short centrifuging' by accelerating to, say, 2500 r.p.m. and applying brake. Pour supernatant into beaker. Repeat if necessary, depending mainly on the quantity of 'fines' present in the residue. Beaker contents (i.e., supernatant pourings) are centrifuged and examined for palynomorphs. If some palynomorphs are present, mount part of the centrifuged supernatant residue as one or two strew slides. If a significant quantity of palynomorphs is found to have been removed by the short centrifuging, return them to the main residue, and repeat the procedure at a higher speed than originally adopted. As mentioned above, some experimentation may be required in order to achieve the desired separation for a particular residue.

Summary of Step 9:

(a) *Deflocculation.* (1) Fill tube with 50% HCl. (2) Place in water bath for *ca* 30 minutes. (3) Centrifuge and decant. (4) Add a few drops of 'Brij 35' and wash/centrifuge three times.

(b) *Mineral removal.* (1) Slightly acidify residue by washing with distilled H_2O containing a few drops of 10% HCl. (2) Half-fill tube containing residue with $ZnBr_2$ solution (S.G. 2.0) and mix thoroughly. (3) Centrifuge at 1000 r.p.m. for 2 minutes. (4) Pipette off organic 'floats'. (5) Critically examine 'floats' and 'sinks' under microscope. (6) Thoroughly dilute and

mix 'float' fraction with distilled H_2O. (7) Centrifuge and wash/centrifuge three times.

(c) *Coarse debris removal.* (1) Cut small square (*ca* 8 cm^2) of 170 μm or 200 μm screening material, and place in small funnel inserted in centrifuge tube. (2) Pipette residue through screen, adding small quantities of 'Brij 35'. (3) Examine screenings for palynomorphs. (4) Centrifuge screened residue.

(d) *Palynomorph 'cleaning'.* (1) Add a couple of drops of 'Brij 35' and of 5% KOH to residue and half-fill tube with distilled H_2O. (2) Heat gently in water bath, constantly agitating the tube. (3) Centrifuge. (4) Half-fill tube with distilled H_2O and place in ultrasonic tank for 2 minutes. (5) Wash/centrifuge three times.

(e) *'Fines' removal.* (1) Add a few drops of 'Brij 35' to residue, agitate, and almost fill tube with distilled H_2O. (2) Centrifuge to selected speed, then brake. (3) Pour supernatant into beaker. (4) Repeat until supernatant remains clear. (4) Centrifuge beaker contents and examine microscopically for palynomorphs.

STEP 10: CONCENTRATION OF PALYNOMORPHS USING ZINC BROMIDE SOLUTIONS

If the palynomorphs are unencumbered with attached organic debris, they should be relatively buoyant. Zinc bromide solutions of lower S.G. than applied to mineral separation (Step 9b) can be utilized, if required, to enhance the concentration of palynomorphs in the residue. It is advisable to commence the operation with a S.G. of 1.6 and then, if necessary, progressively lower the S.G. for subsequent separations by addition of appropriate small quantities of distilled H_2O. S.G. values of less than 1.4 are likely to cause selective incorporation of the heavier palynomorphs in the sink fraction. The procedure requires careful monitoring by examining each fraction microscopically to ensure that palynomorph loss (into the sink fraction) does not occur.

The length of immersion of the residue in the $ZnBr_2$ solution appears to have no adverse effect on the preservational state of the palynomorphs. Some extraneous organic particles appear to absorb $ZnBr_2$ when stood in the solution for about 30 minutes and are incorporated in the 'sinks' by slow centrifuging.

Summary of Step 10:

(a) Centrifuge residue in a 15 ml centrifuge tube and pour off supernatant.
(b) Add 6 ml of $ZnBr_2$ solution S.G. 1.6.
(c) Centrifuge at 1000 r.p.m. for two minutes.
(d) Pipette off 'float' fraction plus 'suspension' (i.e., down to level of 'sinks').
(e) Examine separately both the pipetted fraction and the 'sinks' under the microscope.
(f) (i) 'Floats' + 'suspension': If extraneous organic particles are still present, add 1 ml of distilled H_2O, mix, and repeat (c) and (d).

(ii) 'Sinks': If palynomorphs present, repeat (b) – (e).

(g) Repeat above, if required, until an acceptable concentration of palynomorphs is achieved in the 'float' fraction. Retain the 'sinks' fraction and make a slide (as per Step 12) if any palynomorphs are suspected in that fraction.

(h) Wash/centrifuge three times in distilled H_2O.

STEP 11: FINAL 'CLEANING' OF RESIDUE

This step may be required in order to eliminate hitherto intractable, fine or coarse extraneous particles, the presence of which would tend to mar the quality of the final slides. The following techniques may be employed: (a) sieving; (b) swirling; (c) short centrifuging.

(a) *Sieving.* Very fine meshed, nylon screening material with 10 μm apertures is effective for removal of fine particles, and is most efficiently applied in the final stage of processing when the residue has been quantitatively reduced. If used earlier, the mesh can become clogged and hence of limited effectiveness. Fibres from cut edges of the sieve are sometimes detected in the residue; these can be eliminated by pipetting the residue through the 170 μm or 200 μm screen. Critical microscopic scrutiny of both fractions is, of course, essential. If the fine particles have not been substantially removed by the first sieving, the residue should be washed from the screen and passed through again. Dilution of the residue plus a few drops of the dispersant 'Brij 35' facilitate the sieving operation. Slide mounting (i.e., one or two strew slides) of the -10 μm fraction is advisable.

The following is a summary of the method adopted:

(i) Cut a *ca* 8 cm x 8 cm square of the screening material and insert in small filter funnel placed in centrifuge tube (as per Step 9c).

(ii) Dilute the residue with distilled H_2O and add a few drops of 'Brij 35'.

(iii) Pipette successive small quantities through screen, wash thoroughly, and centrifuge 'washings' for retention.

(iv) Pick up a corner of screen square and wash contents into a clean centrifuge tube.

(v) Make one or two slides of the retained -10 μm fraction (as per Step 12).

(b) *Swirling.* Small quantities of mineral or organic debris that may still persist in the residue can be removed by swirling (= panning) the residue on a small (*ca* 8 cm diameter) watch glass placed on a stable, light-coloured surface.

Stated very briefly, the method is as follows:

(i) Pipette a small amount of residue into watch glass.

(ii) Add distilled H_2O and gently rotate watch glass. The buoyant palynomorphs should be mobilized into the upper fluid layer and can be pipetted therefrom, leaving the heavier unwanted particles concentrated mainly in the lower central part of the watch glass. Any tendency of the residue to flocculate may be arrested by adding a few drops of 'Brij 35' and a drop of 5% KOH.

Palynological application of the time-honoured panning technique was

originally publicized and detailed by Funkhouser & Evitt (1959). We share Gray's (1965, p.570) reservations about its effectiveness in achieving a consistently satisfactory separation and thus do not apply it routinely. Undoubtedly, the method requires practice and manipulative skill, as well as being time-consuming.

(c) *Short centrifuging* has already been described in Step 9(e) and can be applied again, very judiciously, to remove fine light extraneous particles.

STEP 12: SLIDE MAKING

Permanent slides are made of the residue using glycerine jelly as mounting medium. From our own experience, and from verbal and published reports from other laboratories and individuals, we are cognizant of possible deterioration (by drying out) of glycerine jelly slides in the long- or even relatively short-term. Although we have experimented with various other mounting media (e.g., elvacite, canada balsam) in the hope of finding a more durable, optically acceptable alternative, we presently consider that the advantages of glycerine jelly (e.g., miscibility; ideal refractive index *vis-à-vis* palynomorphs; ease of slide preparation) outweigh the disadvantages. Moreover, it is possible, albeit inconvenient and vexatious, to rehabilitate glycerine jelly slides, and, more importantly, to take precautionary measures in the initial mounting procedure (see, for example, Hill 1983). In warm-temperate to tropical climates, it is desirable, even necessary, to store glycerine jelly slides in a cool, air-conditioned situation.

Permanent slides can be made at any stage of the processing provided that the residue is washed to neutrality. If, at any stage of the processing, the technician doubts the efficacy of ensuing steps, at least one slide should be made to record the contents of the residue at that stage. If kerogen studies are required, a slide is made before acetylation or oxidation (Steps 6, 7).

On the processing flow chart (Text–fig. 1), slide making (Step 12) is indicated as a possibility at several stages during processing. It is up to the preparator to determine when processing of a particular sample should be concluded, i.e. when the optimal result is considered to have been achieved.

As glycerine is a medium for fungal and bacterial growth and gelatine, being a protein, promotes growth of many microorganisms, care must be taken during the making of the jelly itself and during the residue slide-mounting. The glycerine jelly stock should be stored in small vials and only reheated a couple of times. Continued reheating can impair the stability of the medium, which should be stored in a cool place.

Permanent mounts for light-microscopic study are made with first-quality glass slides (25.4 x 76.2 mm; *ca.* 1 mm thick) and cover slips (22 x 40 mm; no. 0 grade = 0.9–0.13 mm thick). All slides and cover slips must be cleaned with ethyl alcohol and the slide making performed in a clean and dust-free environment. The technique of slide making requires patience and practice in order to achieve bubble-free slides in which the palynomorphs are evenly dispersed in the one plane.

We have found it advantageous to delay slide making by some 24 hours (i.e., after the processing has been completed) in order that the residue be stood in the anti-bacterial/anti-fungal storage medium (equal parts of 3% Cu_2SO_4 solution and glycerine plus a few drops of thiomersal). The Cu_2SO_4 and thiomersal nullify any possibility of actinomycete growth which has adversely affected some of the slides made directly from purely aqueous residue-suspensions (immediately after completion of processing).

Summary of Step 12:

(a) Have hot plate ready at a low temperature such that the hand, resting on the surface, feels warm/hot. A thin, foil-covered rectangle of fibro-board on top of the hot plate aids even heat-dispersal if there are any 'hot spots' on the plate.
(b) Place a vial of glycerine jelly in a water bath (approximately 50°C) to melt jelly.
(c) Number slides appropriately with a diamond-tipped pencil; clean slides and place on a rack on the hot plate to warm. Clean cover slips.
(d) Centrifuge residue and decant most of the 'storage solution' (see above).
(e) Stir residue with pipette to ensure even palynomorph dispersal and place a drop or two on a prepared slide on hot plate.
(f) Warm (to evaporate off excessive moisture) but do not dry.
(g) Add a drop of melted glycerine jelly via a small glass rod.
(h) Using a clean dissecting needle, mix the residue and jelly thoroughly on central portion of slide; avoid overheating.
(i) Angle the cover slip over the jelly/residue mixture on the slide and, with the aid of a needle, gently lower the cover slip. Allow the mixture to flow to all edges of the cover slip. Warm if necessary.
(j) Invert the slide (cover slip down) on to a slide rack and leave for a few minutes on hot plate to allow the palynomorphs to settle on one plane on the cover slip while the mountant is semifluid.
(k) Remove from heat and leave, cover slip down, for several hours at least, after which slides may be re-inverted and should be left to set for 2–3 days.
(l) Any excess residue/jelly around the edges of cover slips may be removed using a razor blade. Wipe clean with tissue dampened with alcohol.
(m) Seal slides (i.e., along cover slip edges) with gold size or similar clear varnish, using a fine camel-hair brush. Label slides with permanent ink and store horizontally.

STEP 13: RESIDUE STORAGE

The residue remaining after slide making is stored in small air-tight vials in solution of equal parts of 3% Cu_2SO_4 and glycerine containing a few ml of thiomersal.

For dry-specimen picking in connection with scanning electron micros-

copy, it is necessary for the residue to be purely alcohol-based. To change the solution from the normal storage medium to absolute ethanol, wash/centrifuge progressively with first 25% ethanol, second, 50% ethanol, third, 75% ethanol, and, finally, wash the residue twice in absolute ethanol to ensure removal of all H_2O. The reverse procedure is adopted for return of the residue to the normal storage solution.

SUMMARY

The foregoing account covers a range of physical and chemical procedures that may be applied, in various combinations, for the efficient separation of palynomorphs from host sediments. Though laboratory processing is normally undertaken in multi-sample batches, each sample warrants individual assessment and handling to ensure the best possible palynomorph representation and concentration in the final residue.

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PLATE 1

Figs 1, 2. Trilete spores, x750 and x1000 respectively.

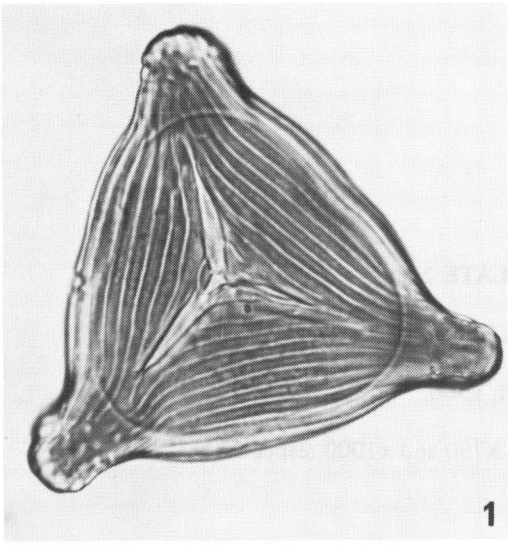
Fig. 3. Monolete spore, x750.

Fig. 4. Polycolpate pollen grain, x1500.

Fig. 5. Triporate pollen grain, x1000.

Fig. 6. Bisaccate pollen grain, x1000.

PLATE 1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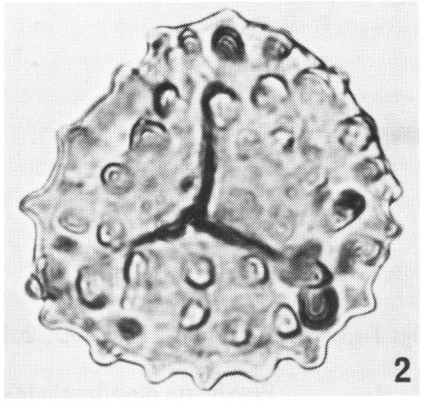
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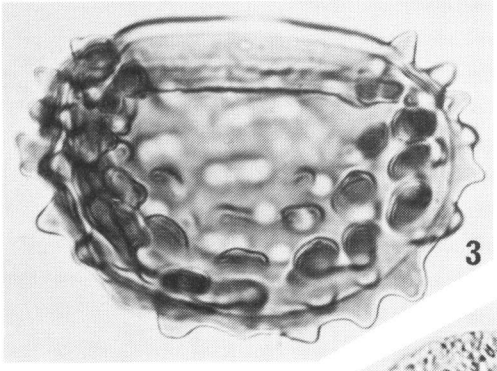
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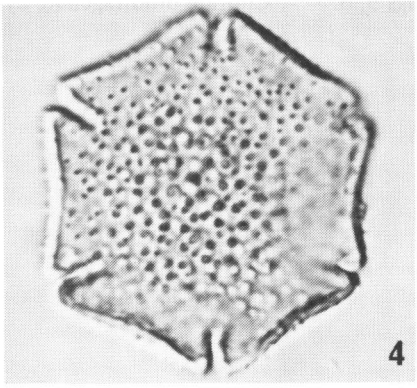
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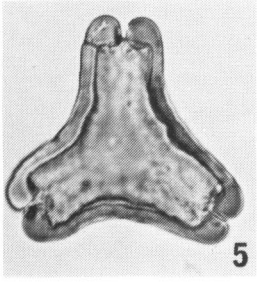
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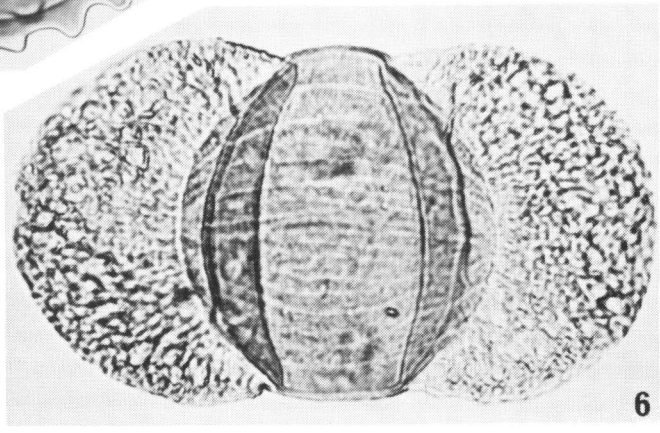

6

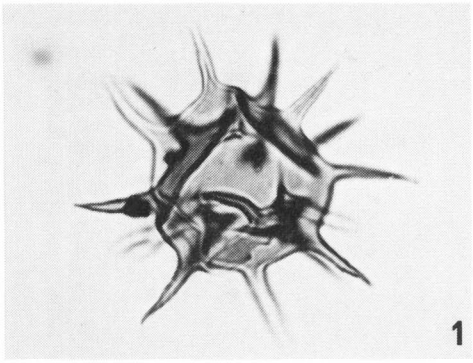
PLATE 2

Figs 1–3. Acritarchs. 1, x1500; 2, 3, x1000.

Fig. 4. Proximate dinoflagellate cyst, x500.

Figs 5, 6. Chorate dinoflagellate cysts, x750 and x1000 respectively.

PLATE 2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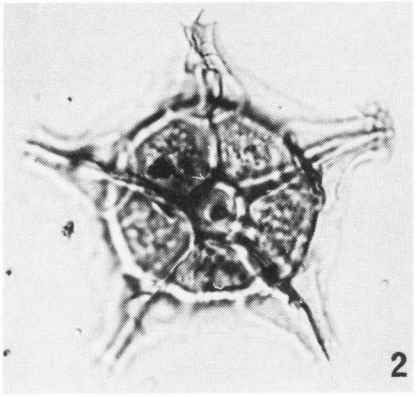
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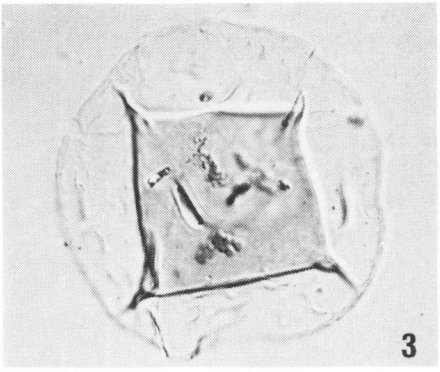
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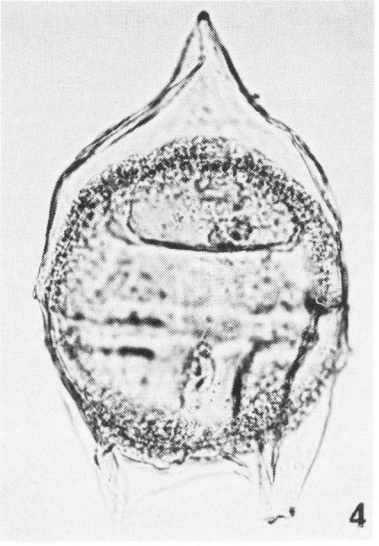
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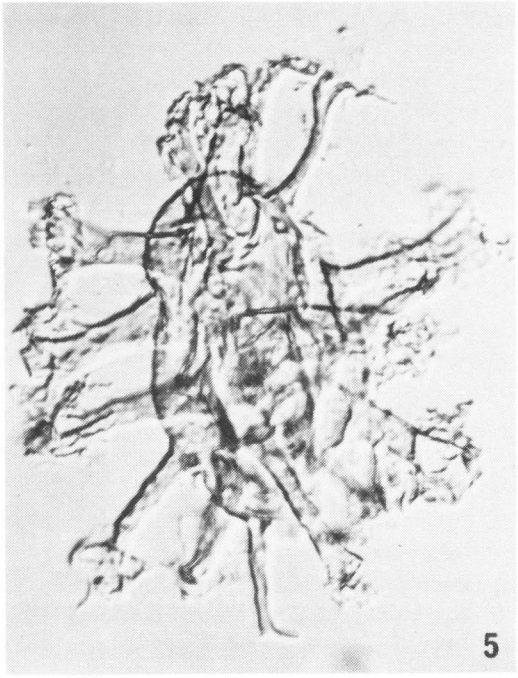
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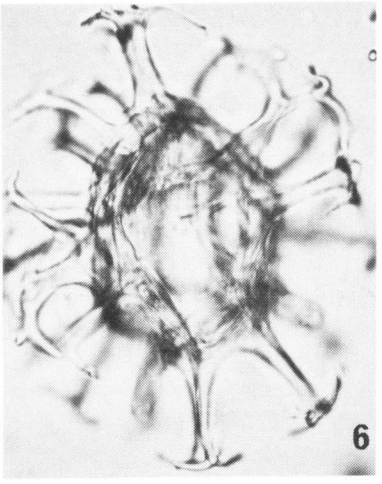
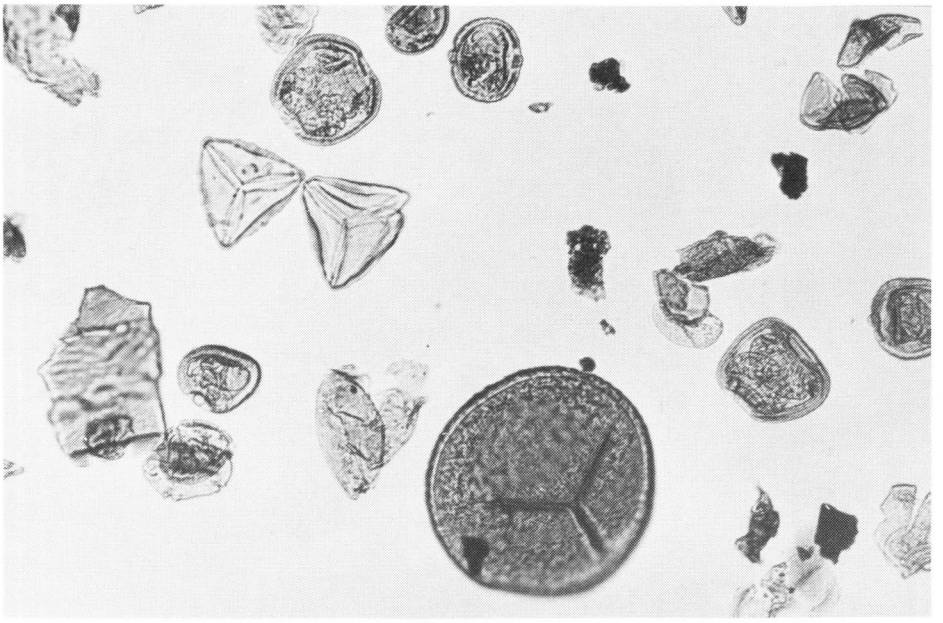

6

PLATE 3
Palynological residues mounted as strew slides

Fig. 1. Spore-pollen assemblage, x360.

Fig. 2. Acritarch assemblage, x450.

PLATE 3



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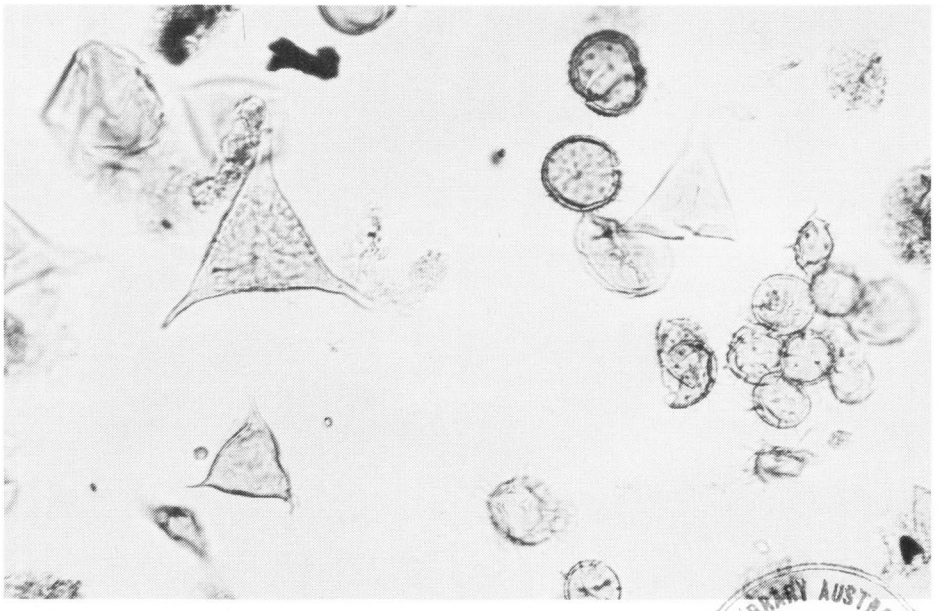

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