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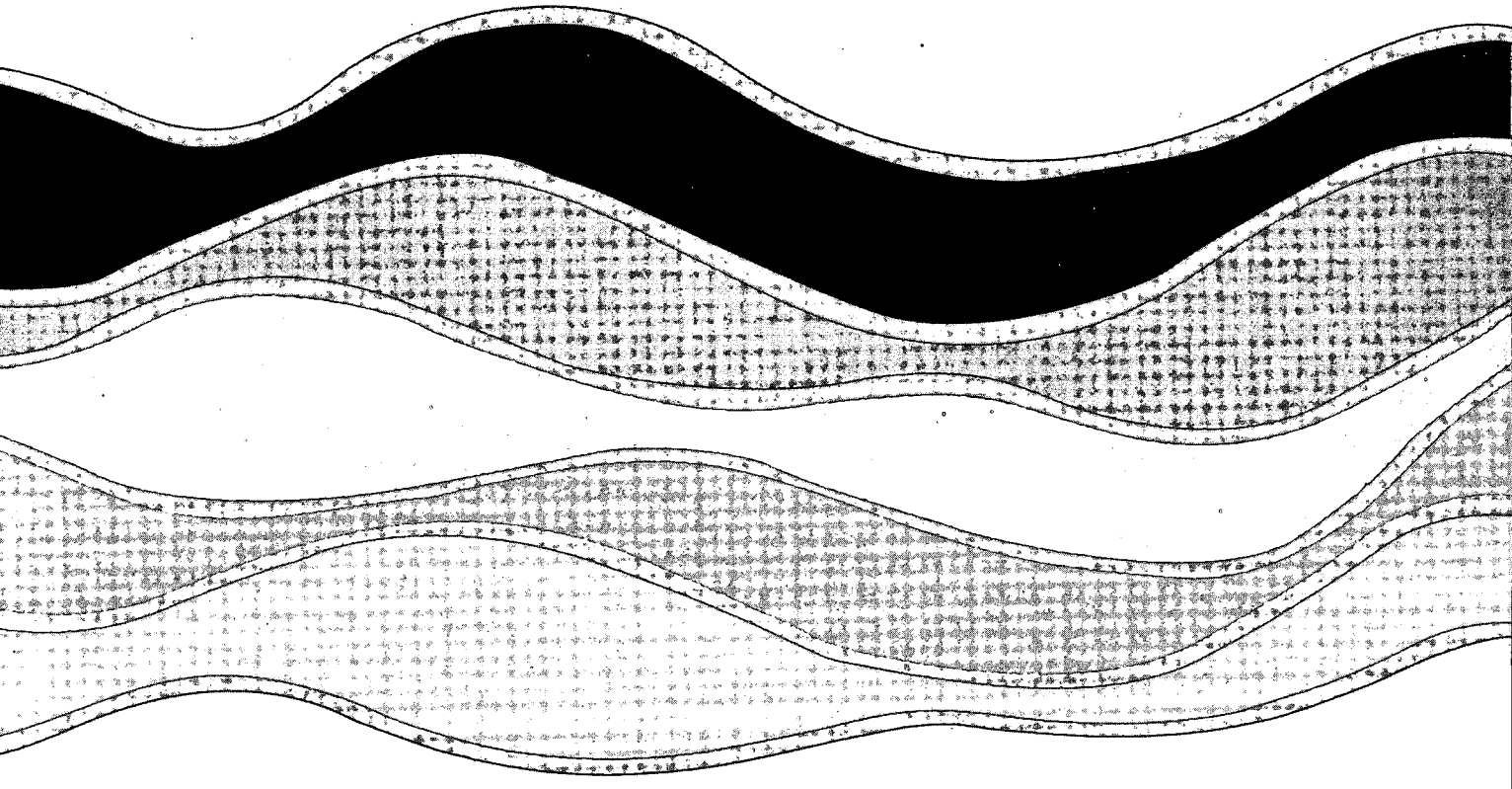

EVALUATION OF ELECTRON MICROSCOPE TECHNIQUES FOR THE DESCRIPTION OF AQUATIC COLLOIDS

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EVALUATION OF ELECTRON MICROSCOPE TECHNIQUES FOR THE DESCRIPTION OF AQUATIC COLLOIDS

by

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MANAGEMENT PERSPECTIVE

This review evaluates the various electron microscope technologies for their capacity to provide realistic characterizations of aquatic colloids. There is a focus on quantitatively important colloids which, before 1990, were considered too unstable to be prepared for realistic descriptions. For this review, a colloid is defined as a particle with a least dimension between 1.0 and 0.001 micrometres, and reference is made to specific roles of colloids in the structure, function and health of both surface water ecosystems and water treatment systems. Minimal perturbation techniques for sample preparation and multi-method approaches to analysis are described and assessed with regard to their contribution towards improved characterizations. The state-of-the-art is defined and some positive future developments are predicted. Accompanying the review is a photo atlas of common aquatic colloids revealed in a state as close to the natural as is possible. This document was prepared at the request of the International Union of Pure and Applied Chemistry.

PERSPECTIVE GESTION

Dans la présente étude, on évalue les différentes techniques de microscopie électronique au niveau de leur capacité de caractériser de façon réaliste des colloïdes aquatiques. On vise les colloïdes dont la quantité est importante et qui, avant 1990, étaient jugés trop instables pour être préparés en vue d'une description réaliste. Aux fins de la présente étude, un colloïde est défini comme une particule mesurant entre 1,0 et 0,001 micromètre, et on mention des rôles particuliers des colloïdes au niveau de la structure, du fonctionnement et de l'état de santé des écosystèmes d'eau de surface et des systèmes de traitement des eaux. Des techniques de préparation des échantillons peu perturbatrices et des approches selon plusieurs méthodes pour l'analyse sont décrites et évaluées relativement à leur apport en vue d'une meilleure caractérisation. L'état des connaissances est défini et quelques progrès positifs futurs sont prévus. L'étude est accompagnée d'un atlas photographique de colloïdes aquatiques courants présentés dans un état s'approchant le plus possible de l'état naturel. Ce document a été préparé à la demande de l'Union internationale de chimie pure et appliquée.

ABSTRACT

Transmission electron microscopy (TEM) can now be used profitably, in conjunction with minimally perturbing preparatory techniques applied in the field, to examine native aquatic colloids of any diameter within the entire colloidal size range (0.001 to 1.0 µm). Morphological observations can be extended to analyses of colloid aggregation behaviour and verified in a multi-method approach by independent complementary techniques. In some cases, size and shape analyses of individual colloids can be supplemented by (1) electron diffraction analyses and by (2) elemental composition analyses via energy-dispersive spectroscopy. Using ultrathin sections of colloid samples embedded in a plastic matrix, one can improve the quality of the measures above and, additionally, one can carry out microchemical analyses by using counterstains which are selective for specific chemical components and which can be visualized unequivocally by TEM. Artifact identification, assessment and minimization can be done systematically, as illustrated by a selection of case studies of some common aquatic colloids. This critical review includes a synopsis of data extracted from the scattered literature on aquatic colloids and presents new TEM information on potentially misleading "particle analyses" of the past. Based on recent TEM analyses, the state-of-the-art, for the characterization of "unstable colloidal particles" is delineated for use by aquatic scientists.

RÉSUMÉ

La microscopie électronique par transmission (MET) peut maintenant être utilisée de façon fructueuse avec des techniques de préparation peu perturbatrices appliquées sur le terrain pour étudier des colloïdes aquatiques natifs de n'importe quel diamètre de toute la plage de taille (0,001 à 1,0 um). Des observations morphologiques peuvent être élargies aux analyses du comportement d'agrégation des colloïdes et vérifiées à l'aide d'une approche à plusieurs méthodes par des techniques complémentaires indépendantes. Dans certains cas, des analyses de la taille et de la forme de chaque colloïde peuvent être complétées par 1) des analyses de diffraction des électrons et par 2) des analyses de la composition en élément par spectroscopie à dissipation d'énergie. À l'aide de coupes ultra-minces d'échantillons de colloïdes inclus dans une matrice en plastique, il est possible d'améliorer la qualité des mesures mentionnées ci-dessus et, de plus, il est possible d'effectuer des analyses microchimiques en utilisant des contre-colorants qui sont sélectifs pour des composants chimiques particuliers et qui peuvent être visualisés de façon non équivoque par MET. L'identification des artefacts, leur évaluation et leur minimisation peuvent être effectuées systématiquement, comme le montre différentes études de cas de certains colloïdes aquatiques courants. Cette étude critique comprend un résumé des données extraites de divers documents sur les colloïdes aquatiques et présente de nouvelles informations de la MET sur des "analyses de particules" antérieures qui peuvent être erronées. D'après les récentes analyses de MET, la fine pointe des connaissances en ce qui concerne la caractérisation des "particules colloïdales non stables" est réservée aux scientifiques du domaine aquatique.

1. INTRODUCTION AND CONTEXT FOR ASSESSING PROGRESS

1.1 General Context

Colloids can be considered as "particles" having a least diameter in the range of about 0.001 to 1.0 um.[1] The significance of the submicron size range is that (1) an appreciable fraction of the molecules of a colloid is located at the boundary region between particle and aquatic milieu, and that (2) a microscope investigation should employ at least one microscope whose resolution permits analyses of the smallest colloids and their surfaces. In addition to their small size, colloids have a "glue-like" or adhesive aspect which gives them complex properties in water. Considerations of these properties from diverse scientific points of view, including coagulation/flocculation and ion binding, are available in the literature.[1,2,3,4,5,6,7,8]

As is increasingly evident,[4,5,9,10,11,12,13,14] colloids play significant multi-faceted roles in the structure and function of aquatic ecosystems and water treatment systems, both healthy and polluted. From the point of view of human needs and frailties, colloids can be major factors in modulating the quality of natural waters, playing roles both positive and negative. To better understand these roles, improved methods of characterization are a necessity.

Electron microscopy (EM) in its various forms is providing an essential technology for realistic descriptions of aquatic colloids of all kinds. This critical review will show that, as artifacts become more readily identified and minimized, EM is increasingly useful in the characterization of colloidal materials previously considered too artifact-sensitive for realistic analyses. The focus will be on the techniques of transmission electron microscopy (TEM) which contribute by providing data on size, shape, porosity, internal

heterogeneity, crystallinity and colloid-colloid associations. As well, TEM is being adapted to permit increasingly sophisticated microchemical analyses, including some for reactivity, and elemental composition analyses.

Among the quantitatively important kinds of colloid under scrutiny by recent technology transfer into the aquatic sciences are the following:

(1) oxyhydroxides of iron and manganese;

(2) fibrils, or linear aggregates of biopolymers rich in polysaccharide;

(3) aggregated fulvic acids;

(4) clays;

(5) water-borne viruses;

(6) picoplankton, or living cellular organisms of colloidal size;

(7) refractory skeletal materials, both organic and mineral.

All of these are treated here, as are some of their natural associations as revealed by minimal perturbation studies. Some of the smallest colloids yield little ultrastructural information on an individual basis, but TEM analyses of their mode of aggregation can be revealing.[15,16]

1.2 Electron Microscopes

The conventional transmission electron microscope[17] is currently the most useful kind of microscope for morphological analyses of colloids and aggregates of colloids. The TEM is similar in purpose to the classical light microscope but with a much greater resolving power; it can provide clear well-defined images of objects which are ca. 1000 times smaller than the smallest objects resolved by a conventional light microscope. A TEM passes an electron beam through the object of study. An image of the object, mainly through differential electron scattering, is carried forward in the electron beam to a viewing and/or recording device. Because imaging depends on the penetrating power of

electrons and because this penetrating power is low for the voltages used in a conventional instrument, an object for study in the specimen plane cannot be thick (less than 0.1 um is preferred). As a consequence of this limitation, most objects are sectioned for examination and an ultrathin section is placed in the specimen plane. The technology for achieving this is outlined in a later section below.

In an up-to-date, well-funded TEM laboratory, there will be a TEM with a resolution better than 0.001 um. Consequently, the choice of which kind of TEM to use for a particular type of sample should be based on factors such as (1) the variety and quality of accessory equipment, (2) the ease of operation, (3) the completeness of the laboratory with regard to specialized electron microscope techniques, and, last but not least (4) the level of skill displayed by the technical personnel.

The laboratory chosen should have accessories which can be coupled to the TEM to permit analyses beyond the purely morphological. One very useful accessory is the increasingly sophisticated energy-dispersive spectroscopy or EDS.[18,19] It allows X-ray microanalysis of colloids, providing information on elemental composition for those elements of atomic number greater than 10; EDS is applicable to the microanalysis of individual colloids in the mid-size range and above. Two other useful accessories are the apparatus of electron diffraction[17,20] for examining crystalline colloids, and the apparatus associated with electron energy-loss spectroscopy or EELS.[21,22] Electron diffraction in association with a field emission scanning electron microscope or FESEM (see Crewe[23] for an introduction to the technology) can be especially useful in analyzing colloids at the lower limit of the size range. EELS is explored elsewhere in this volume.[24]

The conventional scanning electron microscope or SEM[25] can provide a useful correlative technology when used in conjunction with a TEM. A conventional SEM

has a resolution which is not so good as that of a TEM, but it has a much greater depth of focus and requires less complication in specimen preparation. These latter positive features present some advantages relative to TEM (and its requirement for ultrathin specimens) especially in orienting the analyst within large heterogeneous aggregates of colloids where some of the colloids are many times larger than the thickness of an ultrathin section. This problem of the disposition in three dimensions of colloids within a large aggregate and the need for correlative microscopies to provide orientation is discussed more fully below. The capacity of the SEM to analyze extremely thick specimens is a result of the nature of its image formation which is different from that of TEM. In a SEM, a narrow beam of electrons is focused onto the surface of the specimen and is caused to scan it in a regular pattern of lines; the complete response at each instant is used for modulating the signal which governs the image on a viewing screen and/or camera.

There are various hybrids of TEM and SEM which are called scanning transmission electron microscopes or STEM.[26,27] Some are relatively inexpensive in comparison with a high quality TEM and can be fitted with EDS to provide a versatile instrument for the elemental analysis of colloids in the upper part of the size range.[28] Other accessories are contributing to the use of STEM for analyzing relatively (ca. 0.5 um) thick sections.[29]

While a conventional TEM is restricted to analyzing ultrathin sections, it is possible to use TEM to analyze colloids in thick sections through the use of its big brother, the high-voltage electron microscope or HVEM[30,31] whose electrons have extra penetrating power. An HVEM operated at 1000 keV can achieve the same resolution as a TEM operated at 100 keV when using sections 10 to 20 times the thickness permitted by TEM. Specimens of several micrometres thickness can be imaged on a routine basis, but since the images represent volumes, they require special recording and display methods. Because these special methods

are being improved, HVEM has considerable potential as an instrument for the analysis of aquatic colloids, despite its cost.

An instrument of the future having fascinating possibilities is an electron microscope adapted to the visualization of colloids in water through the use of an environmental device (sample compartment) placed within the microscope column. [32,33,34,35]

1.3 Correlative Microscope Technology

To examine large colloids and aggregates of colloids, especially heterogeneous native aggregates whose size extends far into the range for conventionally-defined "true particles" and whose activities lead to the formation of settling particles in aquatic ecosysyems, it can be necessary to employ a battery of correlative microscope technologies so as to bridge the gap between the resolution of the unaided human eye (near 100 um) and that of the conventional TEM (a fraction of 0.001 um). The light microscope can provide a resolution of ca. 0.2 um when used optimally, while permitting an examination of aggregates taken freshly from nature or from controlled experiments. Examinations of gross features can be done directly without any processing or with processing restricted to a simple chemical fixation followed by differential staining. This permits the localization of specific functional groups/families of macromolecules/selected biological materials [36,37] in contexts where the artifact contribution is known. Hayat [38] provides a related guide to the differential staining of biological macromolecules in TEM sections.

There are many useful variations on the theme represented by the standard light microscope which permit improvements/refinements in information yield. Some of the techniques involved have analogs in EM [39] and include phase, interference, darkfield and polarization microscopy [36] as well as the recently developed confocal laser microscopy [40,41,42] and high resolution digital imaging

microscopy.[42,43] Attempts to extend structural analyses well into the submicron range are becoming increasingly more successful through the use of image processing technology.[44,45,46] Some of it is being developed specifically to study living aquatic microbes, with some of the effort being focused on small natural living aggregates of relevance to environmental stresses on surface waters.[47,48] Additionally, biologists are active in studying structural details of aquatic phototrophic organisms in the submicron size range[49] through the use of epifluorescence microscopy[50] on these "living colloids".

A systematic approach to bridging the gap between the near million-fold difference in resolution above (naked eye vs TEM) becomes essential when one wishes to study: (1) the fine details of the three dimensional disposition of different colloid types and their associations in a natural aggregate; (2) the subunit structure/microheterogeneity/porosity of an individual colloid; or (3) the formation of heterogeneous aggregates in experiments. In theory, serial sectioning of embedded colloids would allow one to carry out such TEM morphological studies unaided by accessory microscopes of lower resolution. Practically, however, a blend of correlative technologies is often a necessity and always a blessing to the completion of a successful analysis.[51] The rationale is illustrated by a simplified example as follows; it is based on the need to orient oneself within a volume for the interpretation of the essentially planar images of ultrathin sections, and do it within a reasonably short time frame.

> Let us assume that, to analyze a certain kind of aggregate effectively, one must take sections of 0.050 um thickness through embedded aggregates of 5 um diameter whose heterogeneity is such that one must examine at least 100 examples to draw a meaningful conclusion. Let us further assume that the purpose of the study is to relate details of aggregate morphology to physico-chemical factors, thus requiring 5 different experiments in at least 2 replicates. Thus, in a relatively modest project, one finds a requirement for a photographic documentation of each of 100,000 different sections. This

is clearly a formidable task and one which is attracting the attention of innovators who strive to make the task easier through TEM modifications, new accessory techniques and novel methods of image analysis.[52,53]

A more feasible research strategy, and one which can incorporate innovations as they become available, is outlined as follows:

(1) one can visualize the three dimensional disposition of the larger components of an aggregate using a technology which allows one to look at the entire volume of each 5 um aggregate, even though the resolution may be relatively low (e.g. by light microscopy);

(2) then one can look at finer components with a technology which provides a higher level of resolution while still permitting an appropriately varied selection of views relating to the entire volume of the aggregate (e.g. by SEM);

(3) then, properly oriented with respect to volume, one can employ a selective sectioning approach[54] to provide essentially planar specimens for TEM analyses of all fine details of interest.

This strategy permits an approximate reconstruction of an aggregate which, with an appropriate selection of preparatory techniques for each microscope technology employed, will be close to realistic. One can repeat the work using a different selection of preparatory techniques in a multi-method approach and analyze technique-specific variations in detail for an assessment of artifact.[15] Also, one might wish to employ a TEM-based morphometric analysis,[55] an HVEM approach and some of the novel methods of image analysis mentioned earlier.

The assessment and minimization of artifact can be carried out in a systematic manner through the minimum perturbation preparatory technology currently in development for TEM[15,56] and for SEM.[57] Although the procedures for artifact minimization are increasingly more systematic, there is still a premium to be placed on technical skills. Thus the evaluation of electron microscope techniques for a realistic description/characterization of colloids (and their aggregates) must also be:

(1) an evaluation of preparatory techniques (especially TEM preparatory techniques transferred from the biomedical sciences); and (2) an evaluation of strategies for selecting the most effective blend of preparatory techniques and microscope accessories for a given research goal. The latter evaluation was covered in part in the section above (1.2 Electron Microscopes) and its coverage will be completed in later sections. An evaluation of preparatory techniques was the subject of a recent review [15] and thus will be treated briefly below.

1.4 Preparatory Techniques

1.4.1 An Overview of the "Art" of Sample Preparation

One cannot automatically assume that a specific colloid will be altered adversely by a perturbing mode of preparation. However, it is certainly wise to prepare an incompletely known specimen (or aggregated mixtures/flocs of known and unknown colloids) in a minimally perturbing way if one's goal is to initiate a new literature, that of the characterization of aquatic colloids. The fact that many aquatic colloids show instability in natural waters has been amply confirmed by physico-chemical investigations. [6,58,59,60] It is also evident that the colloidal extracellular extensions of many microbes can be involved in aggregation/flocculation events, and that the morphology and/or aggregation-promoting behaviour of the fibrillar colloids can be altered according to choice of preparatory technique. [12,15,61] Natural organic coatings, which form on particles spontaneously in surface waters, can modulate particle chemistry and behaviour; [62,63,64,65,66,67] thus, interactions at the particle-water interface are of special interest when preparatory technique is of high enough quality to permit morphological analyses near the extreme lower size limit for colloids. The fact that the pursuit of such an extreme level of detail is feasible is attested by recent observations of fibrillar surface coatings [68,69] and on the pore structure of the colloid-water interface material of a hydrated iron

oxyhydroxy-phosphate.[70]

The considerable attention paid to specimen preparation in this critical review is the result of an appreciation, documented in part above, of the current lack of understanding of many limnological materials with regard to structural stability, especially stability changes related to degree of hydration. Because of this concern, there are two guiding principles which one must consider in transferring EM technology from a base in biomedical science to the analysis of aquatic colloids. These principles, which will be developed in a historical context below, before enunciation, give insight into the "art" of selecting the most appropriate preparatory techniques for a given goal.

The best techniques were developed, on a trial-and-error basis, by and for biologists for facilitating the analysis of unstable organic-rich materials, such as parts of living cells. Some are readily adapted to the ultrastructural analysis of colloidal gels and colloidal aggregates rich in minerals, as well as to individual colloids, both inorganic and organic.[15] Originally, they were developed in response to a need to: (1) stabilize complex biologicals for examination of specific features under the harsh dehydrating condition of the high vacuum required by the specimen chamber of a conventional TEM; and (2) reduce the thickness of many kinds of specimens so as to permit an optimal transmission of the electron beam at the accelerating voltages used routinely in the third quarter of this century. The need for stabilization led to the development of modern chemical fixatives[71] and techniques for physical fixation based on rapid freezing,[72,73,74] and also to sophisticated combinations of these two general approaches. The restrictions on thickness of specimen led to the development of embedding resins which retained the integrity of the three dimensional disposition of (at the time intracellular) colloids while permitting the cutting of ultrathin sections by ultramicrotomy.[38]

Large numbers of scientists from many disciplines participated with the biologists for decades in the development of EM preparatory technology and in refining each variation of it for artifact recognition, assessment and minimization. As a consequence of their highly successful efforts, we have a literature on most of the colloids present in living cells and present as extracellular materials on the surface of living cells, as well as a literature on the artifacts created by the application of perturbing preparatory techniques. Additionally, there are scattered contributions in the literature of the earth sciences (see later section for selected contributions) which provide useful ultrastructural information on minerals and on refractory organics much altered from their biological precursors, even though the artifact problem is usually addressed incompletely. In contrast, many of the ontogenetically intermediate colloidal substances/materials in natural waters (ones neither freshly synthesized/exposed nor altered/degraded to a final refractory form) and their natural aquatic associations have no proper ultrastructural literature at all. Thus the situation with regard to the description/characterization of aquatic colloids is one of having to employ different levels of technological effort and skill for any given research, depending on the background knowledge already extant for the material(s) to be investigated. Those who cannot develop well the "art" of technique selection and blending will tend to use time and resources far in excess of the needs for their research.

The historical context above and the uneven way in which technology has developed for the characterization of "unstable" colloids leads to two useful principles to be applied simultaneously:

(A) the literature, especially the literature of <u>cell biology</u>, can serve as an <u>excellent guide</u> for suggestions on how best to analyze a given kind of sample and on how best to seek out and minimize artifacts; but

(B) investigations on most natural aquatic colloids/aggregates, despite assistance from the literature, will have to be complete researches in themselves, including a state-of-the-art treatment of the artifact problem, regardless of increased cost and difficulty, for some time into the future.

The stringent requirements imposed on those who wish to produce realistic ultrastructural descriptions will follow mainly from the specific problems below related to colloid stability, problems which must be addressed now to provide a framework for future research:

(1) fresh biological colloids, once removed from their normal cellular milieu, will undergo a variable series of chemical and physical changes at varying rates, as well as changes in their associations with solutes;[75]

(2) surface active organics, both refractory and fresh, will tend to form coatings on newly arrived particles/colloids, coatings whose physical nature must become better known for an improved understanding of nutrient and contaminant dispersion in surface waters;[5,76,77]

(3) important aquatic mineral colloids in their natural state have been incompletely documented with respect to both reactivity[75,78] and morphology.[70,79,80]

A note of caution is necessary for those aquatic scientists who choose to work in direct collaboration with cell biologists. Those ultrastructural cell biologists involved in perfecting the "art" of artifact minimization will often be found selecting (but deliberately and with good reason) a highly perturbing approach for preparing certain specimens.[73,81,82] This is because they know their specimen so well (from the literature and by experience) that they can predict (and verify later) that their colloid of interest will be visualized realistically, despite the harsh technique, while allowing them to reduce the time and cost of analysis. They may deliberately select a harshly perturbing technique for an additional reason, the fact that it may render more accessible

to measurement some feature which would otherwise be difficult to visualize. This artistic approach has an encouraging aspect because it provides for a possibility that short cuts may be permitted in the aquatic colloid analyses of the future. The drawback is that it may encourage one to take unwarranted and premature short cuts

To complete this overview of the "art" of sample preparation, I present Figures 1 and 2. They illustrate an overview of methods for the preparation of natural aquatic colloids/particles for visualization by TEM. Schemes 1 and 2 of Figure 1 have been presented in detail in the literature.[28,83,84] Scheme 3 of Figure 1 has been discussed by the Nomizu group.[85,86,87] Schemes 4, 5, 6 and 7 of Figure 2 have recently been developed in a paper[56] in which a comparison of all seven schemes may be found. For optimal effectiveness, any scheme chosen should be applied to samples which were perturbed minimally prior to their receipt by the electron microscopist.

1.4.2 Ultrathin Sections

The basic problems of sample stabilization and thickness reduction were solved decades ago through (1) the use of chemical fixatives[71] which preserved colloids/aggregates in a natural state and (2) through gently replacing the water of the fixed specimen by molecules of resin monomer which could be polymerized to produce a hard block of resin-embedded specimen for ultrathin sectioning. The most widely used resins until recently were hydrophobic resins which imposed an extra step in processing; the water of fixed speciemens had to be replaced by an organic solvent miscible with both water and resin monomers,[38] with the solvent being replaced in turn by the resin, thus increasing the level of extraction artifact and exacerbating any artifacts of dehydration.

The availability of a high-quality hydrophilic resin, Nanoplast FB 101,[88,89]

appropriate to the embedding of aquatic colloids,[15,56] has improved this latter situation and opened up new opportunities in the minimal perturbation approach. For extracellular organic colloids and colloids free in the aquatic milieu, both organic and inorganic, the need for chemical fixation is removed through the use of Nanoplast FB 101. Thus, embedding in such a hydrophilic resin can begin in the field immediately after the water sample has been taken.[56,80] For comparative analyses involving several embedding media, both hydrophobic and hydrophilic, with and without chemical fixation, one can consult published strategies.[15] The Nanoplast formulations of Bachhuber and Frosch[89] permit section thicknesses down to ca. 0.010 um, a feature which optimizes the potential for high resolution morphological analyses.[90] This melamine resin has such a fine grain structure that it permits a practical resolution in ultrathin sections of ca. 0.001 um, which is several times better than that of the most widely used epoxy formulations of the cell biologists, including that of Spurr.[91] The clarity of image produced by this improvement in practical resolution is helpful but taking full advantage of it is difficult because of physical effects in the specimen plane which interfere with the interpretation of morphology for details in the size range near 0.002 um.[92] During the polymerization process, there is always a possibility of adverse interactions between the molecules of the embedding medium and the specimen; consequently, any artifacts produced by this interaction are continually analyzed and documented.[88,93]

Morphological analyses are not the only application of a melamine resin for aquatic colloids. Its ultrathin sections are stable enough to permit refined EDS analyses[94] in conjunction with either TEM or STEM. Furthermore, it is suitable for use with some cytochemical techniques (see next section) which permit identification and localization of chemical components.[95]

When sections are viewed by TEM, localized concentrations of heavy elements in the specimens appear darker than the background matrix of the embedding medium. This is because of a superior electron scattering power (greater contrast/electron opacity) relative to that of the light elements making up the resin. This effect has been utilized in the development of counterstains (solutions of heavy metals which have differential affinities for different substances) which can be applied to sections prior to inserting them into the specimen plane of a TEM.[38] This situation is analogous to the use of stains for differential coloring of sections to be viewed in the optical microscope;[37] through the use of TEM counterstains, different colloids rich in light elements receive an artificial increase in electron opacity for improved visualization (and also may become differentiated one from another by taking on different shades of grey according to counterstain uptake). Two of the most useful elements for differentiating biological colloids are uranium[96] and lead;[97] they are often used by cell biologists in conjunction with chemical fixatives which contribute other heavy metals to the image to refine further the level of differentiation. In addition to their general use, counterstains (and also stains employed with fixatives) can be designed so as to be specific to certain families of chemicals and then used with sections which have received no artificial inputs of heavy metal, an experimental tool not to be neglected in the analysis of organic colloids as shown below.

1.4.3 Technology Transfer from Cytochemistry

Technology transfer from cytochemistry should provide many advances in the characterization of organic particles. The potential of this biomedical technology is great, especially when applied to sections in conjunction with the improved correlative optical techniques noted earlier and with EDS.

Cytochemistry is the identification and localization of chemical components (of the cell), with a view to relating functional changes to chemical changes in a morphological context. [36,71,98] Individual techniques can be either chemical or physical and can be applied to either sections or entire particles/colloids mounted on a transparent support (whole mounts). The physical analytical techniques can be either optical or electron-optical [99] while physical preparatory techniques tend to revolve around rapid freezing. [71,72,100] The chemical localization techniques can be conventional, immunochemical or related to in situ enzymatic activity [38,71] and they can be applied to specimens prepared either chemically or physically. All the TEM-based cytochemical technology has its origins in methods which are related to the science of histochemistry, the localization of chemical components on a tissue level. Thus it is based on methods for optical microscopy [101,102,103,104] which are adapted for TEM analyses as needed in an ongoing process. In histochemistry, the ideal requirements for quantification of a localized substance are as follows:

(1) the substance to be measured must be kept in situ;

(2) the fluids into which the specimens must be passed must neither extract the substance nor damage its chemical reactivity to the subsequent identifying reaction;

(3) the reaction used to identify the localized substance should involve a reaction with all of the substance of interest;

(4) the newly "labeled" substance should be readily quantifiable.

There are some stains in general use, both counterstains and stains applied directly to wet specimens, which serve as "markers" for some families of organic polymers likely to be found in at least some surface waters. Examples are: the use of lanthanum as a marker for mucopolysaccharides and glycoproteins; the use of ruthenium as a marker for polyanions such as acid polysaccharides rich

in uronic acid residues; the use of silver as a marker for mucopolysaccharides and proteins rich in cystine. The choice of formulation and mode of use of stains based on these metals is determined by a knowledge of the cytochemical literature and some knowledge of the approximate composition of the sample. The lanthanum and ruthenium formulations are applied to the wet sample prior to embedding [38] whereas the silver formulations are applied to sections. Ruthenium stains employing the mineral dye ruthenium red [105,106] have been especially useful in the description of environmental colloids having a polyanionic character.

Immunocytochemistry or immunoelectron microscopy, a blend of cytochemistry and immunology,[81,107,108] has shown some potential for the characterization of aquatic colloids. Colloids composed of or enriched in sugar polymers containing certain sequences of monomers can act as antigenic determinants. Such colloids permit an immunologist to make antibodies which can be modified to accept a heavy metal component, such as gold, and then used as a molecule-specific stain. A variation on this theme is to couple an enzyme to the antibody and then use the enzyme's activity to generate a localized deposit of heavy metal at the site of the antibody-antigen union. [109]

The immunocytochemical approach to marking specific polymers is proceeding well at the histochemical level for some gelling phycocolloids produced by marine algae. [110,111,112,113] The progress of this work in moving from fluorescent markers (for optical microscope observations) to heavy metal markers is awaited with interest. For living colloids in the submicron size range, principally with medically important microbes, the jump to the highest resolution techniques employing heavy metal markers has been a successful ongoing process extending beyond the scope of this critical review.

Enzyme cytochemistry [71,109,114,115] can become a tool for assessing local

aquatic impacts of protein-rich cell parts recently derived from living cells, especially parts which contain active phosphatases capable of altering the nutrient chemistry of aquatic microniches.[77] Techniques for identifying and localizing enzymes in colloid aggregates are based on the incubation of the sample with an appropriate substrate in a specially designed artificial medium. For example, in one method for phosphatase, phosphoric esters of glycerol are used as the substrate. As a result of the selection of chemicals in the incubation medium, the phosphate ion liberated by hydrolysis is converted into an insoluble metal compound at the site of enzyme action. The buildup of metal at that site can be controlled so that it is large enough to be an obvious marker but small enough to allow a localization in terms of the finest units of morphology. The incubation is done directly with the wet sample, usually after application of a chemical fixative. After the embedding, the three dimensional distribution of the marker metal is analyzed in ultrathin sections with respect to overall morphology. This technology is not without its artifacts and transferring it to the analysis of organic and organomineral colloids from aquatic ecosystems will not be accomplished without difficulties. However, it has been in existence for five decades so there should be no paucity of literature to assist one in making the technology transfer. For living colloids, especially bacteria,[116] a direct literature is already extant.

Despite the exciting potential of technology transfer from the biomedical sciences, one should not neglect the fact that chemistry itself has evolved a use of microscopy which parallels that found in some branches of cytochemistry.[117] The field of chemical microscopy, sometimes called microchemistry, is adept at using various microscopes for analyzing rigid colloids, crystals, high polymers and particle behaviour. In an analogous manner, physics has evolved a microscope technology for pursuing these same subjects. Some of their technology has

contributed to our understanding of the colloidal structural polysaccharides of higher plants, algae, fungi, insects and aquatic invertebrates,[118,119] especially those likely to enter surface waters as refractory debris particles in significant quantities.[75,120]

1.4.4 Cryotechnology

Physical fixation by rapid freezing was mentioned in earlier sections as a sometimes useful alternative[73,81,95,100] to chemical fixation for the TEM or SEM preparation of colloidal materials. While useful in the hands of skilled cell biologists investigating well known biological materials, techniques of rapid freezing place one at the risk of inducing major artifacts of dehydration, such as the extreme shrinkage which can occur to loose aggregates of colloidal organic fibrils.[15] Among the cryotechniques is an extraordinary exception of great potential in all kinds of ultrastructure research. This exception is the time-consuming and costly freeze-etch technique.[74,121,122]

Freeze-etching consists of freezing a sample rapidly enough to vitrify it, mechanically generating a fracture plane through it and then making a metallic replica of the fracture surface (usually following a pre-set level of etching), all the while maintaining the vitrified colloid-rich sample below the recrystallization temperature. The etching consists of a controlled sublimation of bulk water from the fracture surface, so as to place individual colloids in relief. The product of the technique for viewing is an ultrathin replica, created by vaporizing a metal-rich material at an angle onto the fracture face, while maintaining this face below the recrystallization temperature. This replica, when placed in the specimen plane of the TEM, yields a topographical image of a colloid or colloid aggregate, unperturbed by chemical agents or the physical separation phenomena associated with rapid freezing at a rate inferior to

10,000 $K.s^{-1}$. [122] This metallic replica will reveal detail with a resolution as good as that of an ultrathin section made with a hydrophobic resin, and will be more faithful to reality. Because the replica is topographical rather than planar and because the fracture plane may change levels within the sample, image analysis can present complications. [123] Despite its overall complexity and high cost, however, the freeze-etch technique is an ideal alternative confirmatory technique, unrelated to the major standard preparatory techniques normally devoted to particle analysis. Used as part of a multi-method approach in conjunction with ultrathin section analyses, it can permit conclusive decisions to be made about the shape and size and porosity of hydrated colloids when other combinations of particle analysis techniques have been found inconclusive.

1.4.5 Whole Mount Preparations

A whole mount preparation is one in which an entire colloid (or aggregate) is visualized by being placed in its entirety in the specimen plane of a microscope. For the SEM, this presents no difficulty but one is limited to a view of the particle surface; one can fracture a particle to see inside but again one is limited to a view of a surface, the internal one exposed by the fracture. For the TEM, the particle is usually placed on top of an electron transparent support film made of plastic. [38] The electron opacity of the particle puts an upper limit on the size (thickness) of particle which can be examined effectively by transmitted electrons, thus limiting the analyst to particles in the low end of the colloidal size range. Despite this constraint and problems associated with the usually perturbing approaches to whole mount preparation, some progress has been made, most recently by the laboratory of Nomizu.[85,86,87] It is noteworthy that studies of the aggregation behaviour of soil fulvic acids received an impetus from whole mount preparations. [124,125]

An alternative to the traditional TEM whole mount has been explored in detail recently (Figure 2). It is the Nanoplast film technique,[126] which has exciting potential;[56] with it, the smallest native colloids can be added to a fresh Nanoplast resin preparation in such a way as to form a support film having the colloids embedded within. The support film can be made as thin as is needed to allow for a practical TEM resolution similar to that achieved with ultrathin sections. Such a technique for realistic descriptions of aquatic colloids has to be considered promising from the point of view of minimal perturbation. This is because:

(1) it can be applied in the field directly to a sample freshly taken;

(2) it avoids the application of chemical fixatives to the sample;

(3) it avoids air-drying artifacts and the dehydration artifacts which can occur with traditional methods of whole mount preparation;

(4) it can be applied as a practical finishing step in situations which permit the ideal multi-method approach, as delineated by Buffle.[78]

2. THE MOST FREQUENTLY ENCOUNTERED AQUATIC COLLOIDS

The most frequently encountered aquatic colloids thus far described represent a compromise between what actually is common and the specialized interests of the investigator. It is likely that some common colloidal materials have been ignored.

2.1 Non-living Organic Materials

Although the most common organic families of molecules in natural waters are known[75,127] and the more refractory components of the organisms at the base of the food web are known,[120,128,129] there is a lack of detailed knowledge about the extent to which specific molecules relate to given types

of natural waters.[75,127,130] Concomitantly, while some organic colloidal materials can be tentatively identified on morphological grounds by TEM, there can be difficulty in relating morphological details at high resolution to specific arrangements of (and species of) molecules, with exceptions being found among some crystalline organics[131,132] and certain cell parts if visualized at or before the earliest stages of breakdown. For a compendium of cell parts, some general references serve as a good starting point.[128,133,134] Structure, function and chemistry meet at a resolution near 0.001 um and some aquatic colloids have shown themselves to be inherently interesting from ultrastructural, ecological and biogeochemical points of view. When considering the potential significance of colloidal activity in surface waters two decades ago, Breger[135] showed profound insight in the title of his paper used as a closing address to a symposium on aquatic organic matter, "What you don't know can hurt you: organic colloids and natural waters". Since that time, a strong economic interest has also developed.[12]

2.1.1 Fibrils (fibrillar extracellular polymeric substances)

Judging from the volume and variety of literature published, the most interesting of the aquatic organic colloids appears to be the almost ubiquitous extracellular "fibril". This elongate organic colloid rich in high polymers[12,54] is readily recognized in TEM images by its distinctive ribbon-like aspect and greatly increased electron opacity following heavy metal staining.[136] Individual fibrils, whether branched or not, have a diameter typically in the range of 0.002 to 0.010 um; the most common examples are composed at least in part of polysaccharides whose monomeric composition tends to be rich in uronic acid moieties (sugar acids with a projecting carboxyl group). Examples of some morphological varieties, taken from lakewaters, are shown in Figure 3. It is unfortunate that studies to relate fibril morphology to fibril chemistry have yielded little information.

Potential impacts of fibrils on aquatic ecosystems are outlined in Figure 4 whose context can be found in reference [137]. There is evidence that all the phenomena shown do occur, but quantification of their significance has not yet been achieved (with the exception that fibril roles in biofilm formation/ microbe colony formation are vitally important, a topic to be developed later). The value of TEM for the description of fibrils (and the resultant insight into fibril-associated phenomena) is readily evaluated; TEM was and is absolutely essential.

Although fibril research has not progressed as rapidly as had been hoped in the past two decades, enough is known to present a detailed case study pertinent to this critical reciew. To begin, it is given that fibrils represent a family (or families) of morphologically and cytochemically similar colloids which share many common properties. The most evident of these are a biological derivation (from algae, bacteria and plant roots), a limited size range and the presence of carbohydrate moieties in polymerized form. The literature which reveals their similarities is extremely scattered but guides to much of it can be found in two older references. [12,77] Despite generalized similarities, however, it must be kept in mind that fibrils do differ in details such that differences in function and reactivity might be considerable.

Fibrils were noted on the surfaces of a variety of algae and bacteria in the 1960's. [54] During this decade, they were also documented as a component of:
(1) slime layers in streams, in association with microbiota; [138]
(2) organic flocs taken from contaminated waters; [139,140]
(3) highly acid mine waters; [141]
Because of the fear of artifact, many of the conventional TEM observations were confirmed by freeze-etch analyses in the same publication.

In the 1970's, fibrils were found to be a component of the rhizoplane, the

interface between the outer surface of a plant root and the mineral particles of the soil solution. It can be a zone of high metabolic activity, especially when microbes are present, and it influences the chemistry of the adjacent soil zone. [142] The reality of rhizoplane fibrils was demonstrated by both structural and cytochemical analyses of ultrathin sections [143,144,145] and by freeze-etching; [146] they were shown through the use of axenic root culture (no bacteria present) to be produced by root cells, [146] although in nature one would expect also a contribution of fibrils to the rhizoplane by soil bacteria. At this time, fibrils were also identified as the likely agents for contact cation exchanges between mineral colloids and plant cells, [147,148] a role which finds an analogy in lake water. [54] Thus came the link between fibrils and nutrition.

Also in the 1970's, fibrils were shown to be:

(1) a polymeric bridging structure within many microbial biofilms, [149,150] regardless of the biological speciation within the biofilm [12], a bridging structure which could either cross-link microbes or encapsulate them as shown in Figure 5;

(2) a natural adhesive promoting pelagic associations of algae and bacteria in lakes [151], including their associations with suspended abiotic particles;

(3) a functional component of activated sludge flocs, although the evidence tended to be circumstantial; [152,153]

(4) a secretion product of higher plant cells grown as suspension cultures in mineral media, and used for a brief time as a source of sufficient quantities of fresh fibrils for wet chemical analyses; [154]

(5) a common component of many Canadian lakewaters, being detected in lakes of various trophic states and sizes at all levels of the water column from surface microfilm to bottom sediments. [54,136,155]

One can reasonably assume [69] that fibrils comprised the fibers shown by the relatively low resolution SEM images of the 1970's to be a major component of the fine debris of lakewaters. Since those fibers appeared to bind together the individual components of heterogeneous debris particles, then one can infer that the fibrils within them helped to mediate particle aggregation in lakes. Convincing SEM studies of these fibers can be found in the innovative ecological researches of Paerl. [156,157,158,159]

In the 1980's, some attention was placed on quantifying fibrils in lakewater; [84,160] levels up to 7 mg/L were recorded. Research on water treatment systems then became focused more intensively on fibril roles in flocculation. [161,162, 163,164,165] In keeping with the increased emphasis on environmental pollution, especially with regard to recalcitrant organic contaminants, [166] some research emphasis is being placed on possible roles of aquatic flocs as natural decontaminators of surface waters. The focus has been placed on the action of the flocs per se, however, rather than on the interactions of the fibril component with organic pollutants.

2.1.2 Humic Substances

Another group of aquatic organics of interest from ultrastructural, ecological and geochemical points of view is the group, humic substances. [75,167, 168,169] They have become increasingly interesting because of researches in the past decade to relate their chemistry, structure and behaviour to morphological parameters. [83,124,125,170] Their great abundance, refractory nature and interactions with organic pollutants [167,171] demand a greater understanding of their colloidal behaviour. Their nature as colloids, when aggregated, is increasingly amenable to analysis by TEM.

In 1980, Ghosh and Schnitzer [170] produced a model for the macromolecular structure of soil fulvic acids which showed that their colloidal structure was

a dynamic feature controlled by three environmental parameters:

(1) sample concentration;

(2) pH of the system;

(3) ionic strength of the medium.

At a sample concentration of 100 mg/L (other parameters controlled), Stevenson and Schnitzer [124] found five common structural entities, including three classes of giant aggregates amenable to study by TEM. These findings, based on whole mount preparations, compared favorably with those obtained later in analyses of ultrathin sections.[83] The large colloidal aggregates were composed of granules in the ca. 0.002 um size range, organized into dynamic structures as attested by the fact that they could pass ultrafiltration membranes even when the aggregate size was much larger than the pore size. [83] The presence in soils, lakes and groundwaters of moderately large colloid aggregates (0.05 to 0.20 um scatterers) of humic acids has been confirmed by photon correlation spectroscopy. [172] The meaning of these observations is:

(1) humic substances can form a continuum of aggregated particles with widely varying size, in rather fast equilibrium;

(2) ultrafiltration is not always a simple process when applied to waters rich in humic substances and it could become an unreliable one if induced aggregate formation is not controlled.

Further confirmation of the size and shape of humic substance colloids should be done by freeze-etching [74,121] and by Nanoplast embedding techniques [56] in association with experiments on the mechanics of aggregate formation. Soil fulvic acids already present a context for such confirmation and behavioural studies. [83] Some aggregated humic substances are illustrated in the ultrathin section views of Figure 6.

2.1.3 Organic Skeletal Materials and Protein-rich Cell Fragments

Many organic colloids of direct biological origin are readily identified on morphological grounds; these are (sometimes unique) combinations of size and shape and electron opacity coupled with specific indicator details such as surface sculpturing or a geometric arrangement of subunits. Characterization on this basis is especially informative for a variety of refractory skeletal materials, algal cell walls and protein-rich cell wall appendages of small organisms. At the earliest stages of degradation, some protein-rich cell parts (e.g. flagellae and complex organelles) of decomposing organisms are readily classified provided that the fragment's least dimension is at the upper level of the colloidal size range. Subclassification can be carried out in principle on the basis of reactivity (e.g. the presence of hardy enzymes) using the technology of cytochemistry. For an understanding of the potential of such characterizations, one need simply consult the literatures of cell and ultrastructural biology for their characterizations and descriptions permitting the identification of cell parts. One can use some of the general references quoted herein as a guide to this literature, most of which was highly developed some time ago.[128,133,134] Figures 7, 8, 9 and 10 illustrate some organic and organo-mineral skeletal materials and protein-rich cell parts and fragments recorded by the author in his limnological investigations. A very extensive phase of cataloguing is necessary to assess the relative importance of the non-living organic materials, a subject which is in its infancy.

2.2 Living Colloids

Living colloids come in two classes, cells and viruses. Aquatic cells of colloidal dimensions come in two fundamental kinds;[36] these are (1) the prokaryotes such as bacteria and the so-called blue-green algae, and (2) the eukaryotes, such

as the true algae and all other cell types. A full treatment of living colloids extends beyond the scope of this critical review. However, several exciting subject areas of this aquatic research topic have blossomed recently and can be reviewed briefly. The advances concern the viruses (Figure 11) and the picoplankton (Figure 12).

Despite the role of viruses as agents of human disease via water supplies,[173,174] and despite several decades of development of particle counting techniques for viruses,[175] the technology for quantifying viruses in aquatic environments was not fully exploited until recently.[176] Using a new method for quantitative enumeration, the group of Bergh[176] found up to 2.5×10^8 virus-like particles per ml in natural waters. Their counts showed viruses to be present in numbers 10^3 to 10^7 times higher than was previously reported. Considering the virus species which attack and infect bacteria, those with a head size of 0.060 um predominated, smaller than what had been anticipated.

If the viruses are active, the implications of such great numbers are far-reaching indeed. Viral infection of cells at the base of the food web could be an important factor in the ecological control of such cells/organisms, and in turn of their effects on water quality. After this discovery, additional TEM technology was devoted immediately to a search for morphological correlates of viral action on other living colloids in lakewater.[177,178]

Recent atlases of viral ultrastructure show TEM views of both whole mounts and ultrathin sections of common viruses in general.[179,180] Works specialized in TEM examinations and identifications of plant and insect viruses are available[181,182] as are reviews of literature pertinent to algal viruses.[177,178] A critical appraisal of viral taxonomy is found in Matthews.[183]

In the context of "small is important", the picoplankton, or aquatic cellular organisms in the size range of 0.2 to 2.0 um, are now known to be much more important to the biological processes of surface waters than had been believed

until quite recently.[49,184] Understanding their physiological roles in the "metabolism" of surface waters and their relations to viral predators are now important goals of the biological aquatic sciences.[185] Moving from physiology and disease to microbial ecology, another goal is concerned with the secretion of colloidal adhesives (often fibrils) by bacterial picoplankton, adhesives which permit organisms to attach themselves to debris and mineral particles.[186,187] The importance of this attachment is great; in general, aquatic bacteria are more active in biogeochemical processes after attachment to a surface.[188] The sequence of processes whereby attachment influences metabolism, and the subsequent ecological consequences, is an important focus of aquatic microbe research. The mechanics of the attachment process, including the positioning of stabilizing colloids and cross-linking bridges involved in biofilm formation, have been amenable to TEM analyses for decades.[189,190]

The speciation of the algal picoplankton in the submicron range was begun a little more than a decade ago.[191] It can be continued only with the aid of TEM. Concomitant with the TEM-based speciation research, limnologists and ecotoxicologists anticipate using TEM as a tool for health and toxicological assessments[192] by correlating changes in the chemistry and morphology of sensitive picoplankton with specific environmental stresses. Some picoplankton with distinctive morphological features[193] are shown in Figure 12.

2.3 Mineral Colloids and Mineral-Organic Associations

Mineral colloids have been studied extensively and well by microscopy for decades.[194] The general literature on mineral colloids is large and readily accessible.[195,196] However, its EM literature is essentially about the minerals per se, featuring "cleaned" minerals free of naturally associated materials or particles of a pure mineral which had no aquatic context. The experience of

this author in examinations of surface water colloids is that the minerals are typically eroded/coated/irregularly fractured/aggregated with dissimilar colloids to a considerable extent, such that their classical features are not always evident in samples which have been perturbed minimally. While the focus of this critical review is on realistic descriptions of both individual colloids and natural colloid associations, it must adhere to its theme of descriptions/characterizations of aquatic colloids in their native state in aquatic ecosystems. This means that, with a few noteworthy exceptions, our concerns herein are restricted to mineral colloid associations with organic coatings, inorganic coatings, microbiota, extracellular enzymes, colloidal ion exchangers (such as fibrils) and other mineral colloids. Where pertinent to the theme of "native state", individual colloids will be considered. In this context, mention will be made of morphological parameters related to the growth of hydrated colloids into hydrated "conventional" particles, including crystallinity changes.

The topics above are increasingly amenable to development by way of TEM and EDS analyses of minimally perturbed samples, with electron diffraction and cytochemistry as useful adjuncts. In response to recent transfers of high technology, the aquatic sciences are creating a literature on such topics. The situation with respect to unperturbed mineral colloids has definitely advanced for both water[28,94] and sediment samples.[80,197] The necessary concepts and technology for continued progress are increasingly refined [15,56,78,107,121,198] and there is no lack of interesting experiments to do and hypotheses to test.

Two topics of potential interest worthy of mention are: the genesis of mineral colloids in water by biota; [198,199,200] the synthesis and organization of mineral-rich skeletal structures by biota. [201] However, they take us far enough into the biological literature to extend beyond our scope; studies of natural associations between mineral colloids and microbes are readily accessed in the bacteriological and phycological literatures. [198,199] With regard to

mixed inorganic/organic flocs, there are good experimental systems available to analyze the mineral contribution to mutual flocculation between minerals and cells/organics.[161,202,203,204] Increasingly amenable to TEM-based analyses are molecular interactions at the mineral-microbe interface.[198,205]

Discussed below are some specific studies on mineral colloids in their approximate native state in aquatic ecosystems. These studies were chosen because they involve various combinations of improved sampling, improved preservation, selective staining and the use of EDS or electron diffraction on a "per colloid" basis. The remarks will focus on a few mineral colloids (containing Fe, Mn, P, Ca, Si and Al) which play important biogeochemical roles in aquatic ecosystems.[206]

2.3.1 Inorganic Associations With Organic High Polymers

In 1988, Stone[207] made the following statement in reference to the interactions of mineral particles with surface active aquatic compounds of high molecular weight. "Whether such organic compounds are spread over entire mineral surfaces or are found in patches on mineral surfaces is not known. The nature of this surface coverage can be expected to have an important impact on the availability of mineral surface sites for chemical reaction." In making this statement, he is providing a comment on the limited capacity of conventional chemistry to detect the detailed distribution of a coating material at a mineral surface. There is, however, a microscopical/limnological technology which does not have this limitation, at least for the case of organic fibrils (Figures 3 and 4). The possibility of elucidating the disposition of fibrils on various particles in mixed aggregates, using TEM, was demonstrated some time ago,[68,69] although a systematic use of the technology on the major types of mineral particles has not yet been attempted. The mode of attachment of fibrils to an

inorganic surface was demonstrated two decades ago [190] when the nature of the binder was identified as acid polysaccharide. [189] Considering that organic fibrils are rich in acid polysaccharide, [54] the facts above are relevant to the finding of Davis [208] that acidic functional groups on natural organic matter are important in complex formation at the mineral surface. TEM clearly has potential to assist the efforts of environmental analytical chemists in the analysis of high polymer organic coatings.

The relations between metals/mineralization in an aquatic milieu and active biopolymers (extracellular enzymes and/or secreted colloidal ion exchangers) as studied by TEM [77,209] is a specialized field which is growing in sophistication. [198] Considering the enormous variety of organo-metallic species in natural waters [75,210] and their various reactivities with respect to biota, [211,212] TEM should play a larger role in describing the relations between metals, biopolymers and mineral formation.

In conjunction with metal-organic interactions, inorganic coatings on minerals is a subject of interest to many scientific disciplines. In aquatic environments, the abundant hydrous iron and manganese oxides can act as scavengers of (and eventual sinks for) heavy metals [213] including toxic ones. In a pollution context, such incorporation of inorganic substances by mineral surfaces is a scientific field connected to socio-political issues. In this context, some novel attempts to analyze iron and manganese oxyhydroxide colloids have begun using TEM and STEM-EDS [80] applied to diagenetic colloids collected <u>in situ</u> from sediments and embedded directly in Nanoplast for ultrathin sectioning. [214,215] While the genesis of inorganic coatings is an important research area on its own, such genesis in the presence of organic coating agents should become a major research topic within the area.

2.3.2 Iron-rich Colloids

Iron-rich particles in freshwaters have become much better understood in recent years. [216] Concomitantly, the growth of hydrated mineral particles from molecules is increasingly amenable to microscopical analyses. While investigating the iron-rich materials of lakewaters, Laxen and Chandler [217] found stable iron-rich particles in the submicron range. In 1984, Tipping and Ohnstad [218] were able to assess the colloid stability of such particles. This put a focus on describing the genesis of iron particles in lakes [78] and the speciation of such particles. [28,219] As a result, through the use of minimally perturbing techniques and a multi-method approach, the formation of a mineral colloid and its aggregation "growth" into "true" particles was analyzed in detail for an amorphous iron-rich material found in the redox transition boundary layer of a lake. [28,70, 78,79,94] In this study, near-spherical iron-rich colloids (globules) in the range of 0.05 to 0.31 um were shown to be composed of subunits in the nanometre size range. Accessory techniques [220] showed the iron component to be close to one-half Fe(II). A provoked flocculation of colloid-rich lake water showed the iron-rich globules as participants in the formation of loose aggregates which were well into the size range for true particles (by sticking to each other and to other colloids, both organic and inorganic). Some examples of the globules are shown in the micrographs of Figure 13, accompanied by a representative spectrum. The relationships between globules and their aggregates is diagrammed in Figure 14; globule subunit structure was recently investigated with regard to iron partitioning phenomena in relation to filter fractionation. [70]

EDS showed individual iron-rich globules to contain also the elements Ca and P (identified as PO_4 by laser mass spectrometry microprobe analysis) in the mean molar ratios Ca:Fe = 0.19 and P:Fe = 0.25. As a generalization, individual globules came in three morphological varieties, one of which was

rare and another of which was strongly associated with other mineral particles as a mixed colloid. A bulk approach to analysis showed many small aggregates to be Fe/P/Ca/Si/Al and others to be Fe/P/Ca/Si. However, the Si/Al and Si components of the mixed colloids involved could be shown to be inadvertant associations.[94] Individual Fe/P/Ca globules, with or without adhering clays or silicates, in turn could be part of larger heterogeneous aggregates containing recognizable patches of organics, clays, calcium-rich colloids and silicon-rich structures (some of which were identifiable by their morphology as bits of the silica frustules, or mineral walls, of diatom algae). The globules, either alone or in aggregates, had a surprisingly restricted size range; however, the lower limit was a function of anomalous filter behaviour.[70] The cutoff filter, a standard filter of 0.45 um used by limnologists,would retain globules down to a diameter a little less than 0.05 um but would pass globules smaller than 0.04 um at the filter flow rates used at that time. This phenomenon was investigated in detail so as to use flow rate comparative studies as a tool to analyze the behaviour of iron colloids,[70] a subject treated in detail elsewhere in this volume.[9] To summarize, in globule-rich samples, colloidal iron of average dimensions much smaller than 0.04 um was found in experiments not based on capture by a 0.45 um filter; these appeared to be singlets and multiples of the near 0.002 um granules which were the substructural units of globules and the basis for the internal porosity of globules. In this context, it is interesting to note that Schneider and Schwyn[221] have proposed for iron hydroxides a hexameric basic"building block" which has a diameter near 0.002 um.

Morphological analysis of aggregates revealed a feature often seen in studies of distinctive colloids; despite the tight association of globules in many aggregates, a globule-globule association was readily recognized as such. Thus, <u>the effect of perturbation was to increase the size of the aggregate but not the size of individual globules</u>.

Figure 13 illustrates part of a combined morphological-EDS analysis of amorphous iron colloids and makes evident the "separation effect" gained through the use of ultrathin sections to localize and identify individual colloids within an aggregate. In this case the section thickness is less than the thickness of most of the colloids of interest, and, of course, colloids above and below those of interest have a minimal contribution to the complexity of the image (by being in preceding and succeeeding sections). Those colloids rich in heavy elements are of course "self-staining". Once an aggregate in section view is mapped for heavy elements, it can then be mapped for biologicals (sometimes) through the application of counterstains.[28] The combination of spectral and morphological detail achieved through the use of ultrathin sections cannot be achieved in analyses of multimicron-sized aggregates visualized as whole mounts.

Crystalline compounds of iron, and amorphous iron-rich materials containing crystalline components in the lower part of the colloid size range, are amenable to analyses by a combination of TEM, EDS and electron diffraction. Such analyses are currently being pushed to their limits[222] and promise to extend our knowledge of crystal nucleation in natural waters. They should also help to refine our understanding of the "amorphous" state for iron compounds, a subject of growing interest.[223] Sectioned iron compounds from aquatic environments have permitted a morphological resolution of ca. 0.001 um in Nanoplast[70,222] and permit high grade spectra from colloids as small as 0.04 um diameter during routine use of TEM-EDS. Diffraction patterns potentially useful for "fingerprinting"can be obtained from iron-rich crystals with a diameter below 0.005 um.[222] This last figure may seem surprisingly good to some, since electron diffraction parallels X-ray diffraction, but one must remember that electrons interact about 10^6 times more strongly with matter than do X-rays (and that the identification of near-nanometre crystals in an ultrathin section is not difficult).

Even with variable mixtures of several different iron-rich compounds in a complex organic matrix, one has a possibility to selectively identify specific crystalline iron compounds in samples whose size is extremely small in relation to the sample size requirements of conventional wet chemistry. This capacity of TEM-based technology for research on microcrystals within complex mixtures certainly merits further exploration and has already been well utilized in studies of iron-rich magnetosomes, the crystalline colloids of magnetite found within the cytoplasm of some aquatic bacteria.[224,225] These intracellular crystals of cuboidal-to-octahedral shape have diameters mainly in the range of 0.040 to 0.050 um and are arranged in chains. Evidence from cell remagnetization studies indicates that individual bacteria possessing these chains have properties of single domain ferromagnets. TEM has contributed greatly to confirming that the individual magnetosomes contain iron in the form of magnetite.

In 1983, the crystal habit and magnetic domain structure of individual magnetosomes was analyzed[226] using a field emission electron microscope.[227] Each colloid was determined to be a single crystal with a hexagonal prism shape truncated by $\{111\}$ planes; the lattice spacings agreed with those of magnetite. A subsequent study in 1984[228] by high resolution TEM with electron diffraction on the growth and development of magnetosomes revealed that the 1983 story[226] was not complete. In the later study, direct evidence was presented for both crystalline and non-crystalline phases within individual magnetosomes. It led to an interesting hypothesis on the mechanism of biogenic magnetite formation, one whereby magnetite crystallization involves hydrated iron (III) oxides as non-crystalline precursors.

The isolation and detailed characterization of native iron colloids is intimately tied to an improved understanding of minimal sample perturbation.

Mention was made earlier of colloids of 0.05 um diameter (Fe/P/Ca globules) being captured at the upper surface of a cutoff filter whose pore size was nine times larger than the colloid. The basis for this is found in colloid "stickiness" phenomena which allow some variables in the filtration process (mainly flow rate) to promote aggregate formation at the filter surface.[9,70] This filtration-induced creation of "true particles" was investigated further in relation to the (increasingly misleading) dogma that the 0.45 um cutoff filter of limnologists separates particles from solutes, regardless of colloid instability problems (and in a conceptual framework often oblivious to the existence of colloids). This "particle creation" artifact is treated in depth in another chapter of this volume.[9] A brief and to-the-point commentary on the current use of 0.45 um filters by limnologists/oceanographers (applicable also to the 0.7 um filters of water quality analysts) was published in 1990.[229] Well-presented arguments for considering colloid fractions separately from solute and true particle fractions can be found in the literature since the 1970's.[230] When this concept finally takes hold in aquatic science laboratories, progress in research on iron colloids, and colloids in general, should leap forward. As a final comment on the need for considering colloids as entities presenting special problems in characterization and definition, look at the magnification marker in the figures showing photomicrographs. It shows 0.45 um as a bar which is larger than the colloids of interest, thus defining them as solutes by conventional limnological/oceanographic thinking.

2.3.3 Manganese Oxyhydroxides

Research on manganese colloids in sediments is being done in parallel with investigations of iron-rich colloids in the same sediments.[80,215] One of the goals is to ascertain just what is meant by the term "ferromanganese colloid" when it is applied to a natural colloid "system". For example, is the system

composed of colloids rich in Mn plus Fe, or is it a two-component system consisting of Mn colloids coexisting with Fe colloids? There has been some technological success at achieving the means to make such distinctions.[214,222] In concert with this effort, there has been some success in demonstrating a partitioning of cations between Mn and Fe in natural aquatic colloid systems.[80]

Manganese oxyhydroxides are ubiquitous constituents of aquatic ecosystems and, within the sediments, they are believed to impact on the cycling of toxic trace elements. Despite their obvious importance, little has been learned of their native colloid structure and physico-chemical properties.[215] This lack of information is a result of (1) their dilution in the sediment matrix coupled with (2) the lack of a minimally perturbing separation technology for isolating them until 1989.[80,215] At present, one can effect an in situ collection of diagenetic Mn colloids, spatially separated with respect to Fe colloids and readily dissociated from much of the organic matrix, through the use of sheets of an inert material inserted vertically into sediment,[215] and left there for varying periods of time. The preparation for TEM can be initiated directly after removal of a sheet,via Nanoplast embedding applied to the Mn-rich film adhering to the sheet.[80,214] The narrow, minimally perturbed film is large enough to be split into subsamples for multi-method analyses involving optical microscopy, SEM, TEM, EDS and many of the conventional techniques of wet chemistry.

The genesis of Mn colloids can involve an active participation by bacteria.[200] Through the use of bacterial cultures capable of making colloidal Mn from added manganese sulphate, Ghiorse and Hirsch[209] made the following observations of relevance to aquatic colloid studies. The bacterial cells produced fibrils extending from their surface into the aquatic milieu; colloidal Mn oxide particles formed in association with the fibrils, apparently through enzyme action.

2.3.4 Colloidal Phosphorus

Phosphorus in the form of phosphate is a major nutrient, often a limiting one, which can be a contaminant when too abundant in surface waters.[206] Changes in the availability of P to biota can cause dramatic alterations in aquatic ecosystems. Nearly, two decades ago, Lean[231] demonstrated that a colloidal phosphorus material, rich in an organic component, might be significant in nutritional exchanges between plankton and lakewater. Later, Francko and Heath[232] distinguished two kinds of complex phosphorus compounds with regard to nutrient phosphate release in lakes; those sensitive to enzymatic hydrolysis and those sensitive to photodegradation. In 1984, Persson[233] described a promising physical technology for the separation and characterization of phosphorus-rich colloids from lakes and rivers. Recently, Ridal and Moore[234] conducted a re-examination of the measurement of dissolved organic P in seawater. They showed the colloidal fraction to comprise 20 to 50% of the total "dissolved" organic phosphorus. The moment is propitious to develop a mode of analysis of colloidal P based on TEM, EDS and correlative technologies. With this idea in mind, one must remember that the Fe/P/Ca globules described earlier, the nucleic acid-rich viruses and some nucleic acid-rich picoplankton all qualify as colloidal P (and even in many laboratories as "dissolved" P when they pass 0.45 um filters).

2.3.5 Biogenic Calcium and Silicon

The biogenic nature of much of the abundant kinds of aquatic particulates rich in calcium[206,235] or silicon,[133,206] and the concomitant variations in ultrastructure, complicate morphological analyses of submicron-sized versions of them. However, the importance of Ca and Si activity to the modulation of biological processes in aquatic ecosystems[201,206] merits a greater research effort on their aquatic colloidal species. Descriptions of the most abundant particulate species of Ca and of Si, including some biogenic kinds, can be

found in mineral atlases for earth scientists; [194] some are distinctive morphologically but their distinctive aspects tend to disappear for eroded fragments in the lower part of the colloidal size range. They are readily analyzed by EDS, however, because both elements have peaks in EDS spectra which are distinguished readily from each other and from the most abundant "heavy" elements whose particulate species are sampled from surface waters. Their natural associations with mineral colloids of distinctive appearance are currently under analysis by minimal perturbation techniques. [94]

Colloidal $CaCO_3$, calcite, presents a mineral surface in natural waters which is effective in adsorbing many organic compounds. Photosynthetic cells, through removal of CO_2 from their immediate aquatic milieu, can precipitate calcite at the cell-water interface and initiate the formation of calcium-rich particles whose fresh calcite surfaces are readily exposed to secreted organic molecules. Some roles of organics in determining the nature of mineral species formed at the surface of coated calcite are reviewed in Morse. [235] Analyses by TEM and EDS of these phenomena can contribute to their understanding.

Silicon, in terms of the compound silica, is relatively unreactive chemically in many surface waters. However, Si is important in the cycles of (often abundant) diatom algae which assimilate it for the synthesis of their mineral cell walls (frustules). [133,206] Silicon occurs in soluble forms (silicic acids) and in particulate forms as well as adsorbing to/complexing with other substances. In the author's experience, silicon-rich materials sampled from freshwaters are difficult to recognize in the lower part of the true particle size range when identification is based purely on morphology; colloidal fragments derived from them are even more difficult to recognize. There are two exceptions to this generalization: (1) some of the clay minerals [194,196] as shown in the next section; (2) the spectacular frustules of many varieties whose colloid-sized

fragments are readily identified by their regular geometric aspect (provided that the fragments are not too small). Figure 15 illustrates frustules in different stages of fragmentation/degradation, including one approaching colloidal dimensions and accompanied by its EDS spectrum. In the case of the living example shown in cross section with its cell structure intact, one can see evidence, at the frustule-water interface, of the high polymer organics which are usually associated with frustule mineral. While diatoms are the most noted examples of single-celled organisms which make silicon-rich structures, they are not alone. Of relevance to this critical review, there are examples of aquatic bacteria which can precipitate silicon-rich materials; some examples are summarized briefly in Beveridge. [198]

2.3.6 Clay Minerals

For several decades, there has been a literature available on TEM descriptions of mineral structure *per se* and much of this has featured clays. [194] This literature contributed to an understanding of both the ultrastructure and activity of clay colloids. [196] However, the literature was not concerned with minimal perturbation techniques, having little need for them in descriptions of rigid materials and having only recently developed a need to visualize better the coatings on minerals, and other colloid-colloid associations. Simultaneous with this TEM contribution to mineralogy, there developed a biological literature focused on clay-microbe-fibril associations. The researches were concerned with the effects of the soil environment on the morphology of submicron biota, [236] and with the spatial relations between clays, microbes and high polymer organics, [145] including cytochemical analyses of ultrathin sections. [143]

Submicroscopic studies of soils *per se* [237] and of clays, [197,238] however, have begun only recently to receive the detailed attention that they merit. A few advances are especially exciting in the context of this critical review. Using

a hydrophobic embedding resin and placing emphasis on correcting problems of sample perturbation, the Bennett group [197] have made considerable information gains through TEM analysis of clay sediments in ultrathin sections. They were able to show, for sediments of unconsolidated high-porosity marine clays visualized at high resolution, that the pore profiles of these sediments had aspect ratios which approach 1.0. The pore profiles of consolidated low-porosity clays were characterized by aspect ratios (length-to-width ratios) which approach infinity. Their data suggested that sediment fabric is a function of both the characteristics of the constituent particles and the physical/chemical environments of deposition. They had some success with computerized image analyses carried out on SEM and TEM micrographs of sediments. Initial results appeared promising for quantifying fabric parameters and providing a statistical basis for fabric descriptions. An extension of this research [32] employed a TEM environmental device [35] in a preliminary way as a tool for the analysis of hydrated clay colloids. This innovative approach may soon be complemented by new developments in scanning tunneling microscopy which permit atomic resolution microscopy of surfaces immersed in water. [239] Adding to all this recent technology transfer are the initial attempts to analyze clay colloids embedded in hydrophilic resins. An example of a minimally perturbed clay particulate is shown in Figure 16 accompanied by its EDS spectrum.

3. WHERE ARE WE NOW? - A SUMMARY

3.1 The Behaviour and Interactions of Aquatic Colloids

Individual colloids within an aggregate and individuals which participate in experiments on aggregation can be characterized on the basis of shape, size, native electron opacity, internal heterogeneity, porosity and elemental composition. Some organics can be further characterized with counterstains and molecule-specific

"markers", while crystals can be further characterized by electron diffraction. Aggregates can be classified according to shape, size distribution, proportion of minerals, proportion of crystals, evidence of biota present, degree of packing, nature and frequency of non-cellular biologicals, evidence of occlusion phenomena and the frequency of association between numerically-important colloids. An expanded treatment of aggregate substructure for organics was published recently.[15] In analyzing aggregation, one is not necessarily restricted to examining before-and-after situations; cryotechnology should be applicable to the analysis of stages of aggregation when these are relatively fast in comparison to fixation/embedding times. Applied spinoffs from high-resolution analyses of adhesion or "stickiness" phenomena are likely to include:

(1) improvements in the use of membrane filters in fractionation of colloid-rich natural waters (and, potentially, wastewaters);

(2) improvements in our understanding of occlusion phenomena whereby natural colloids bind contaminants in such a way as to produce misleading chemical analyses for the contaminants.

3.2 How to Minimize the Artifact Problem

To maximize the amount of information which one can obtain from an aquatic environmental sample, it is necessary to analyze the sample in a state as close to the native state as is possible. To achieve this goal, one must handle and process the sample as little as possible.

The minimal perturbation approach consists of:

(1) taking the sample in the most gentle manner possible;

(2) isolating the material of interest in the most gentle way possible, and doing so in a manner so as to keep the number of processing steps to a mimimum;

(3) avoiding, if possible, concentrating the sample --- otherwise let the water body concentrate the sample for you, as one can do with lakes by conducting a

search beforehand for the stratum most rich in the colloid of interest;
(4) avoiding sample storage --- otherwise store it for a duration as short as possible (< 1 day);
(5) preparation for EM which involves as few steps as possible and a choice of steps which minimizes colloid instability artifacts;
(6) using extraordinary means to ensure colloid stability prior to the final embedding step, means such as isolating oxygen sensitive colloids at the oxygen tension level where the colloids are found.

Through the use of the minimal perturbation approach, one can present to the TEM a sample so close to its native state that one can analyze profitably the smallest units of structure at high resolution. While artifacts can appear, they can also be identified, assessed and minimized in a systematic manner. These latter topics are pursued through the use of multi-method approaches.

Multi-method approaches allow one to surround the problem of artifact. If several different chemical/physical/biological techniques are applied in colloid analyses, and all point to the same conclusion, then one is likely to arrive at a correct conclusion. In the context of EM analyses, if several different modes of colloid preparation for microscopy all show the colloid to have a least diameter of "x" and if an independent verification by freeze-etching is in agreement with "x", then "x" is the correct answer. This use of a multi-method approach can overwhelm all objections to the analyses of colloid instability which are based on the attitude that extreme difficulty equals impossibility.

Some successes in employing an optimal combination of multi-method approaches with minimal perturbation approaches were described in earlier sections. General strategies are available in the literature [15,56,78] and the author's opinion of the state-of-the-art is outlined below.

3.3 The State-of-the-art

The state-of-the-art in the realistic visualization of aquatic colloids is:

(1) the application of the Nanoplast film technique (see Figure 2) for preparing small colloids and small aggregates of them directly after sampling; [56]

(2) the application of the Nanoplast embedding technique for preparing large colloids and large aggregates of them directly after sampling; [70,80]

(3) independent verification by freeze-etch technology of any contentious finding;

(4) the most gentle sampling technique available, used without sample storage and employing extraordinary precautions when necessary. [70,78]

4. DATA ANALYSIS AND INTERPRETATION

The nature of the potentially available data is as follows for unit colloids:

(1) shapes

(2) sizes and size distributions

(3) porosity

(4) native electron opacity levels, an indication of heavy element levels

(5) elemental composition on a "per colloid" basis

(6) crystallinity identification/fingerprinting on a "per colloid" basis

(7) acquired electron opacity, an indication of selective affinity for heavy elements

(8) internal differentiations in addition to porosity, for large complex colloids.

The nature of the potentially available data is as follows for aggregates:

(1) shapes

(2) sizes and size distributions

(3) degree of packing

(4) relative frequency of colloid types

(5) distribution of colloid types with respect to the surface of an aggregate

(6) natural associations between colloid types.

For experimental work, a classification scheme based on static images can be used in principle to interpret dynamic processes. Time sequence experiments on colloid aggregation and aggregate ageing are feasible with cryotechnology. It is already evident that identifying individual colloids within a compact aggregate is not necessarily a serious problem for image analysis techniques. For example, work on the perturbation of globular Fe/P/Ca colloids, in the presence of other colloid types in the same sample, allowed one to visualize changes in aggregate morphology while permitting conclusive identification of individual Fe/P/Ca globules, even disintegrating ones. [28,70]

For describing large complex shapes, in the case of individual colloids, one can employ the techniques of morphometry developed in the past for biologists and now being expanded for soil and clay scientists as they turn to the use of ultrathin sections. Automated analyses [240,241] may be useful for some kinds of samples provided that sample preparation is compatible with a minimal perturbation approach. For large complex aggregates, HVEM and the use of multiple correlative microscope technologies present a difficult but feasible aid to data collection. To analyze the data of complex samples, systematic computerized approaches are evolving rapidly to assist with three-dimensional reconstructions, EDS spectra and electron diffraction patterns. Also, a recently purchased electron microscope will arrive with a battery of useful computer apparatus and software.

Despite the aid given by computerization, and the promise of a more highly evolved technology to come, photomicrography will remain the backbone of aquatic colloid descriptive studies well into the future. Since recognizing what to photograph (within the myriad of images displayed by the microscope's viewing screen) is a function of skill and experience, there will persist an "art" of particle analysis well into the future. With it will follow the great losses in time required to bring the skills of a laboratory up to the level permitting a

competent practice of the art. There is an urgent need to break these skills down into their individual components and create systematic procedures based on them. In keeping with an urgent need to become more systematic, the colloid investigator must increasingly relate his interpretations to earlier works which included uncontrolled and unassessed artifacts. The urgency is heightened by the fact that data on environmental samples containing unknown distortions from colloid unstability artifact is data employed to assess pollution problems which impact on public health and highly valued natural resources.

5. VALUES AND PARAMETERS IN THE LITERATURE

5.1 The Point of View of a Biological Electron Microscopist

The ultrastructure literature of cell biology is rich in information on the colloids within living cells. Chemically, these include proteins, nucleic acids, polysaccharides, lipids and mineral inclusions. Structurally, these include granules, membranes, fibrils, tubules and certain hybrids of these. Most of these substances and structural entities are readily degraded in surface waters. There is also a wealth of information on the colloidal components of extracellular structures, including refractory organic cell walls, mineralized cell walls, various coatings on small organisms, secreted scales, a plethora of types of skeletons of small plankton and the extremely fine layered walls of prokaryotes. The principle refractory molecules of concern to fresh waters are cellulose, lignin, chitin, pectin and perhaps the protein-polysaccharide and protein-lipid hybrids of prokaryote cell walls. In oceanic waters, the molecules derived from extracellular structures would include the principal algal polysaccharides. In addition, there are the tannins released by many kinds of plant and algal cells. While the cell biologists will take shortcuts with the minimal perturbation

approach, they arrive at sound conclusions about colloid systems when they confine themselves to their specialty. In the case of those colloids, both organic and inorganic, whose genesis as an extracellular material is directed metabolically, the picture painted by cell biologists is likely to be a good one. Included in these comments on their literature are the closely related ultrastructure literatures of specialists in bacteria, protozoa, algae, fungi and the lower animals.

5.2 Suggestions for Revising Values and Parameters

The literature of limnology and oceanography is seriously distorted in its considerations of colloidal phenomena. As remarked upon earlier, this is a result of their out-of-date working assumption that all aquatic materials can be defined as either particles or solutes (no colloids) by causing natural water to flow through a standard cutoff filter of, usually, 0.45 um pore size (often without assessing flow rate effects on colloid stability and adjusting the rate accordingly). There is great potential for an acceptance of colloids and an enlightened view of colloid behaviour making a valuable contribution to the limnology and oceanography of the future. TEM could play a lead role in making this contribution, both with descriptive work and monitoring work on water fractionation procedures.

The literature of soil science is finally evolving in the manner of the literature of cell biology. Hopefully, it will be guided so as to avoid unnecessary contentious issues. Through the use of ultrathin sections it is leaping forward and perhaps now is the time to introduce Nanoplast techniques and freeze-etching.

Given the profound changes in technology produced in the past decade, one must question the value of past researches which impinge on our understanding of aquatic colloids. The mineralogists who contributed a vast literature on mineral colloids have placed most of their emphasis on cleaned minerals whereas it is

increasingly evident that mineral surfaces in an unclean (coated) state play major roles in the biogeochemical processes of aquatic ecosystems. The cell biologists and their cousins in the biomedical fields did many things well; yet, despite their prodigious efforts, the picoplankton were ignored until the late 1970's and the abundance of aquatic viruses was not realized until the late 1980's. With regard to the soil scientists, I have yet to see my first view in their literature of a minimally perturbed, dehydration-sensitive, clay colloid photographed at high resolution.

It would be wise to use the literature of the past as a guide, particularly the more highly evolved versions of it, but only as a general guide to form hypotheses. Every observation of importance to the success of a new research should be checked. Even publications which led to obviously correct conclusions may be in error with regard to specific details. Priorities for revising published values should be set by the various specialists as opportunities arise to make a scientific contribution (with the exception of the high priority research on water fractionation already suggested for limnology/oceanography which should begin immediately).

6. FUTURE NEEDS

Science needs to create a proper literature on the structure, composition, activity and behaviour of aquatic colloids in relation to the structure, function and quality of aquatic ecosystems. Specific attention in this literature should be placed on the cell-mineral interface and on those aggregation processes in the water column which lead to sedimentation and to occlusion phenomena.

Technical advances are needed, and appear to be already forthcoming, for reducing the time necessary to survey sectioned samples. Time is currently the single biggest factor inhibiting research on aquatic colloids. Reducing the

skill components of analysis and interpretation to a system of standard routine procedures is needed to change the artistic component of electron microscopy to a more systematic one. Success in this endeavor will in turn reduce the constraints to progress imposed by the excessive time requirements of the past. With further regard to standardization of methods, the approach of "tuning" the minimal perturbation technology to the specific properties of the natural water sample under investigation must evolve in sophistication.

Immediately, there should begin a reassessment of past "particle analysis" data and derivative information currently utilized by environmental managers and modellers in the service (and potential disservice) of public health and environmental conservation. There are reasons why some pollutants are modelled as solutes by some groups and as particle-bound substances by others --- and the reasons do not reflect well on aquatic scientists. This reassessment should be followed up by a positive research effort to correct the basic problems. In this regard, the inappropriateness of the two fraction scenario in water fractionation schemes must be addressed; natural waters do contain colloids in addition to solutes and true particles.[9,229]

Last but not least, the effect of native colloids on the bioavailability of toxic substances and nutrients should be pursued with greater vigour; TEM can be a great aid to such research.

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FIGURE LEGENDS

Figure 1. An overview of methods for the preparation of natural aquatic colloids/particles for visualization by TEM; Part 1, reprinted with permission from Perret et al. [56]

Figure 2. An overview of methods for the preparation of natural aquatic colloids/particles for visualization by TEM; Part 2, reprinted with permission from Perret et al. [56]

Figure 3. Varieties of fibrils sampled from lakes. Figure 3a shows some of the thickest fibrils documented to date. It and Figures 3b,c,d all show fibrils in counterstained ultrathin sections. Each micrograph was reprinted with permission from Massalski and Leppard [155] where details of preparation may be found. The bar and all subsequent bars represent 0.45 μm, which represents the pore size of the traditional filter used by limnologists/oceanographers to separate particles from solutes. Note that fibril length cannot be measured in ultrathin sections; a fibril has enough curvature to move in and out of the section.

Figure 4. Fibrils, fibril components and fibril aggregates; their potential impacts on geochemical, physico-chemical and biological processes in natural waters. Quantification of these impacts is in its infancy; some will be important at least some of the time. Reprinted with permission from Leppard.[137]

Figure 5. Microbial biofilms. These and all subsequent micrographs show counterstained ultrathin sections unless stated otherwise. Figure 5a shows a fibrillar biofilm matrix in which the fibrils form direct cross-bridges between adjacent bacteria. Figure 5b, reprinted with permission from Leppard,[68] shows a bacterium residing in a cavity whose boundary is made of oriented fibrils in a locally differentiated portion of the fibrillar matrix.

Figure 6. Lacustrine humic substances. For Figure 6a, the humic substances were concentrated before being embedded, so as to aggregate them. Note the weak fibrous aspect which is commonly seen. Figure 6b shows undegraded fulvic acid, concentrated prior to embedding to provoke colloid formation. This fulvic acid, whose smallest granules are ca. 0.002 μm in diameter, was isolated from metal contaminated water; no heavy metal counterstain was necessary for this preparation.

Figure 7. Microcrystalline organic cell walls which can degrade to submicron size fragments. The wall of a healthy algal cell prepared according to ref. 54 is shown in Figure 7a, while Figure 7b shows a recently discarded wall prepared in the same way. Figure 7c shows a degraded wall fragment aggregated with minerals and fibrils, reprinted with permission from Massalski and Leppard.[155]

Figure 8. Extracellular patterned wall layers which can become detached from cells. Figure 8a shows patterned fragments of wall from a lacustrine microbe, reprinted with permission from Massalski and Leppard.[155] Figure 8b reveals an analogous situation for a eukaryote alga.

Figure 9. Some examples of the large variety of protein-rich materials encountered in survey work on surface waters. Figures 9a,b,c present, in order, some biological membranes, a relatively undegraded bit of muscle and some differentiated extensions of bacteria. In a quantitative sense, these are minor aquatic components. Figure 9c is reprinted with permission from Massalski and Leppard;[151] its colloids of interest are described in detail in ref. 134.

Figure 10. Partially degraded extracellular skeletal structures. Figure 10a is reprinted with permission from Massalski and Leppard.[155] Figures 10b,d were prepared according to ref. 54. Figure 10c is a whole mount preparation.

Figure 11. Viruses aggregated within the fibrillar matrix of a slime particle. This virus aggregate, prepared according to ref. 54, was sampled from the water column of a lake. Included are several incomplete viruses.

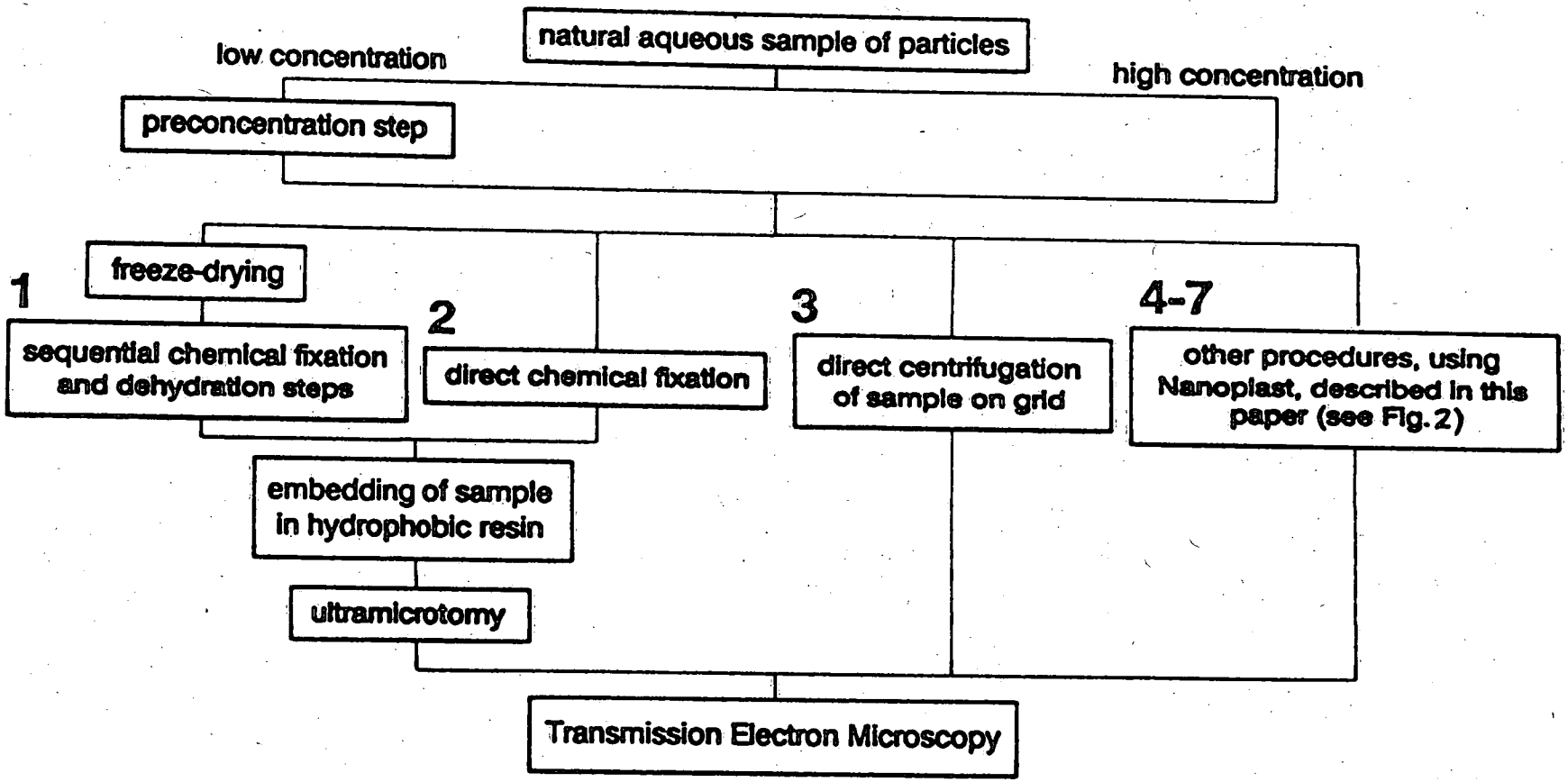
Figure 12. Pelagic picoplankton. Figure 12a shows a cell with a distinctive type of wall, reprinted with permission from Leppard et al.[193] Figure 12b reveals different aspects of the cell contents of a prokaryote picoplankton by showing different section planes through a given species prepared according to ref. 84.

Figure 13. Amorphous iron globules from the oxic-anoxic interface of a small eutrophic lake. Figures 13a,b owe their electron opacity entirely to the native heavy elements within them, and are reprinted with permission from Leppard et al.[28] A typical spectrum from the globules in Figure 13a is presented in Figure 13c. The principal Fe peak illustrated is the Kα peak centred near 6.4 keV. P is near 2.0 and Ca is near 3.7 keV.

Figure 14. Size classes of iron-rich globules, globule aggregates and globule parts found in an iron-rich lakewater fraction originally isolated on a 0.45 μm filter. This Figure was adapted from ref. 28.

Figure 15. The fragmentation of the silicon-rich cell walls of diatom algae. A whole mount of a fragment of frustule with its EDS spectrum is presented in Figure 15a, while Figure 15b shows a frustule in an early stage of fragmentation. Compare the whole mount of the dead cell of Figure 15b with that of the sectioned "live" cell of Figure 15c. This latter Figure is sectioned so as to show the overlap of the two frustule walls when encasing the algal cell contents. Organic polymers are visible at the outer frustule surface in Figure 15c but the section reveals very little of the geometric pore structure.

Figure 16. Clay particles. Figure 16a illustrates the detail that one can document in a whole mount of a suspended clay particle near the colloid-true particle size overlap. The inset shows its EDS spectrum with obvious peaks for Al, Si and K. Figure 16b shows an aggregate of submicron particles, including clay colloids, and is reprinted with permission from Massalski and Leppard.[155]



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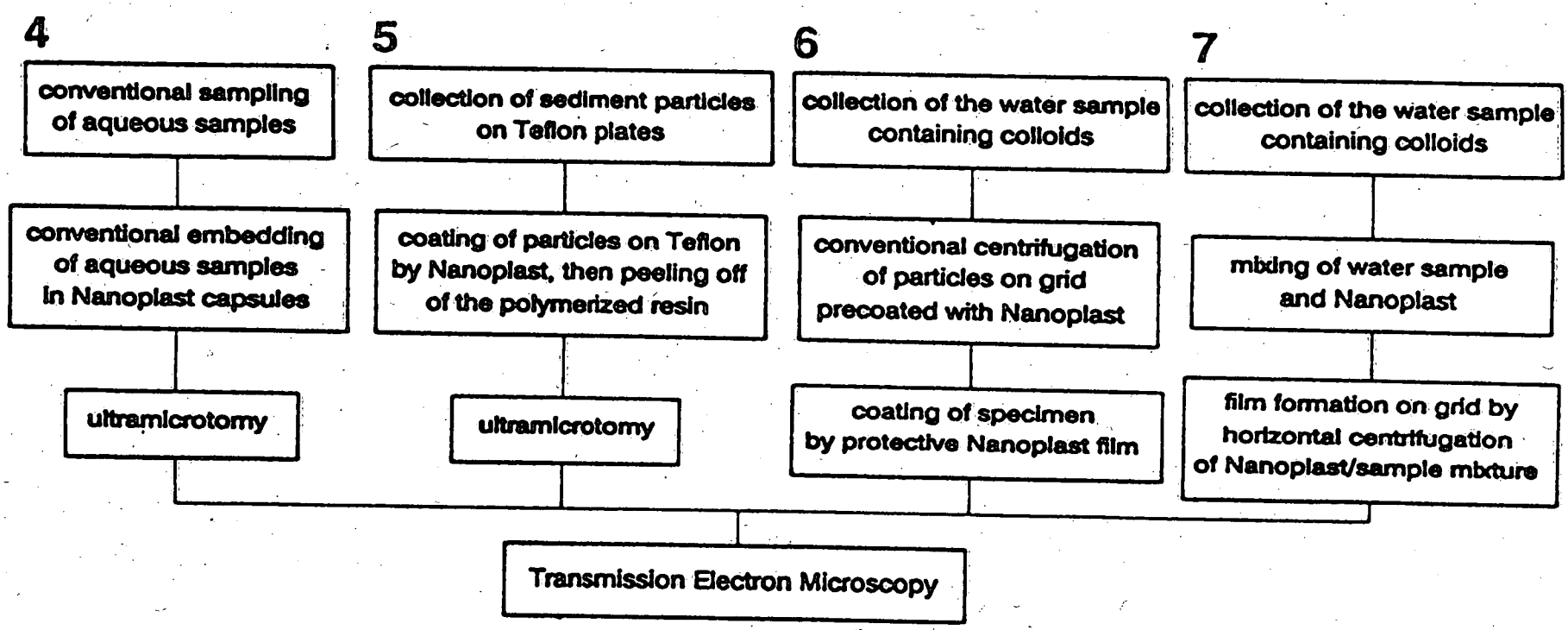
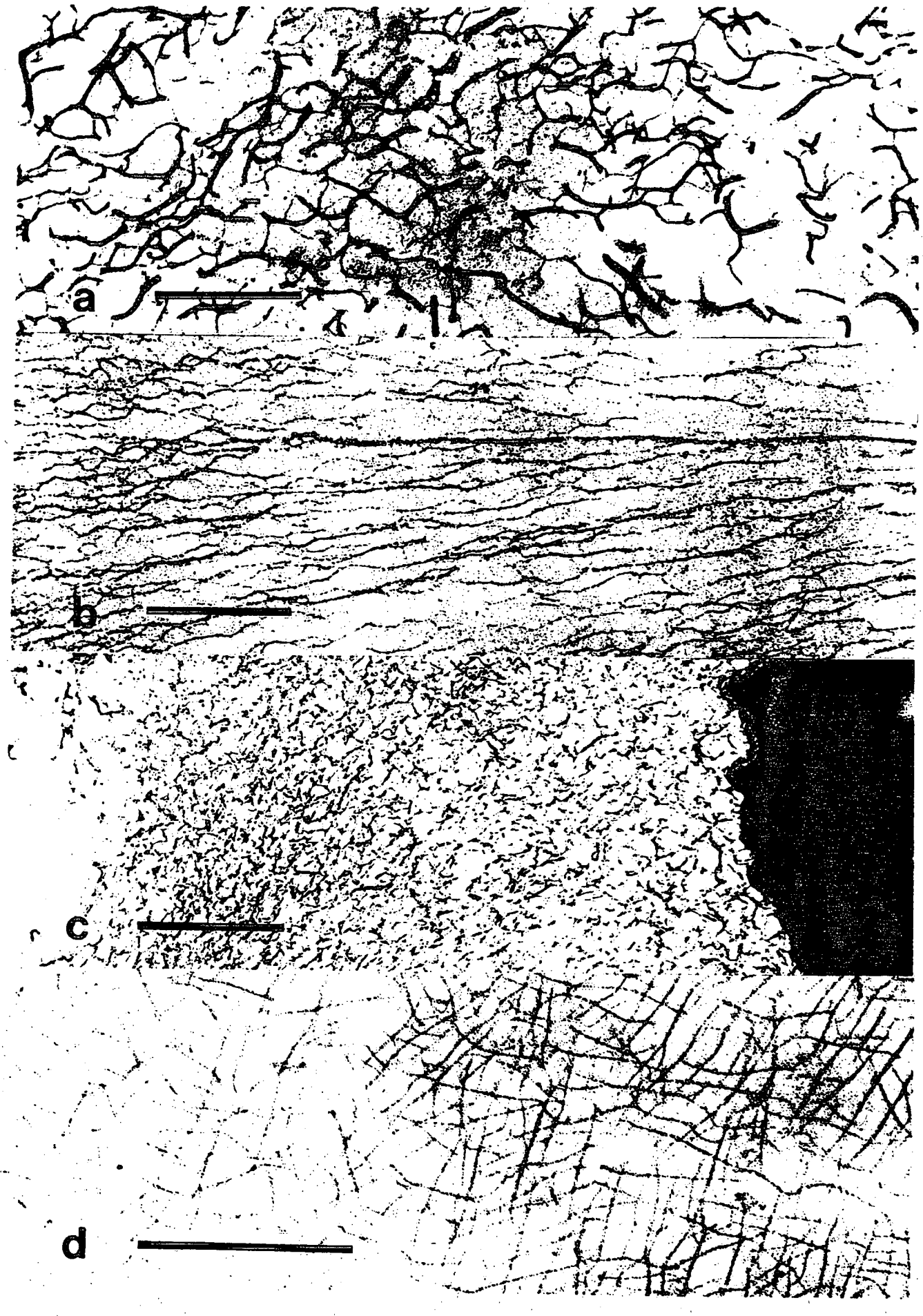


Figure 2. An overview of methods for the preparation of natural aquatic colloids/particles for visualization by TEM; Part 2, reprinted with permission from Perret et al. (1991).


a
b
c
d

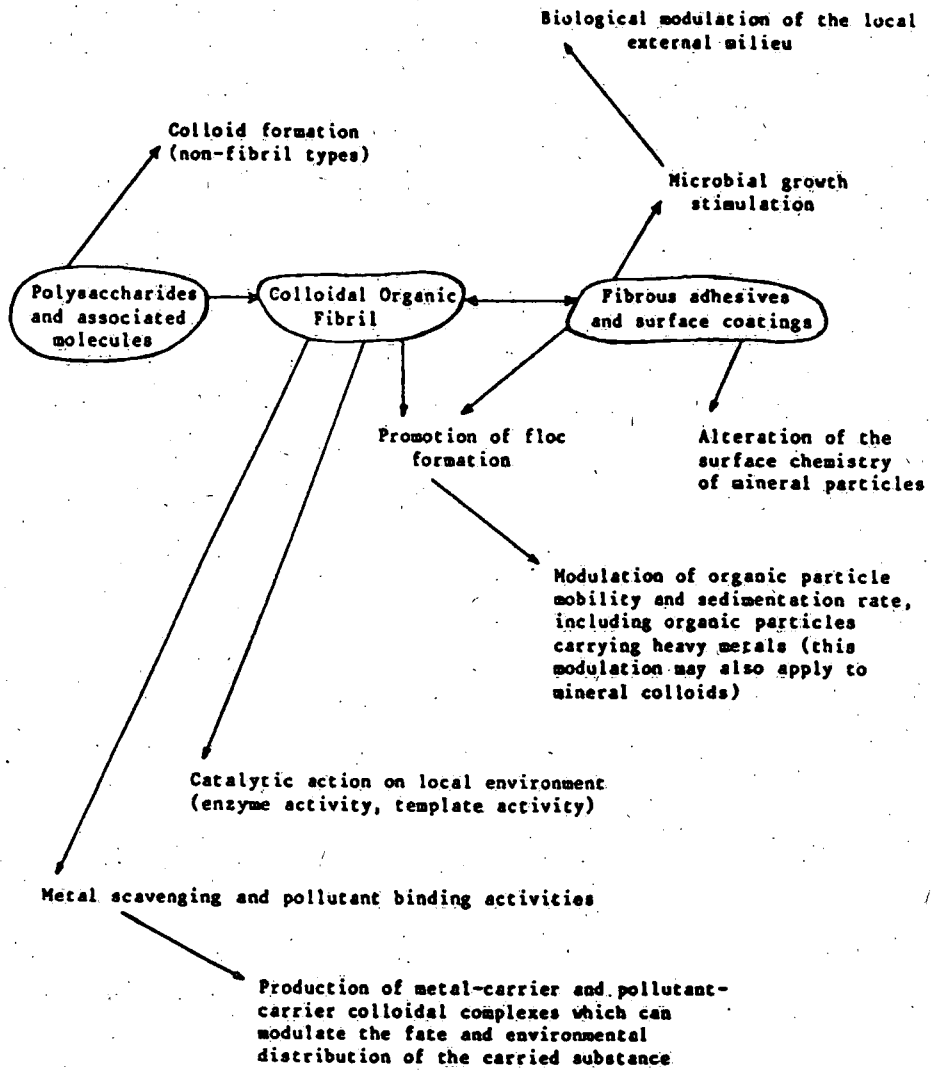


Figure 4. Fibrils, fibril components and fibril aggregates: their potential impacts on geochemical, physico-chemical and biological processes in natural waters. Quantification of these impacts is in its infancy; some will be important at least some of the time. Reprinted with permission from Leppard (1985).


b

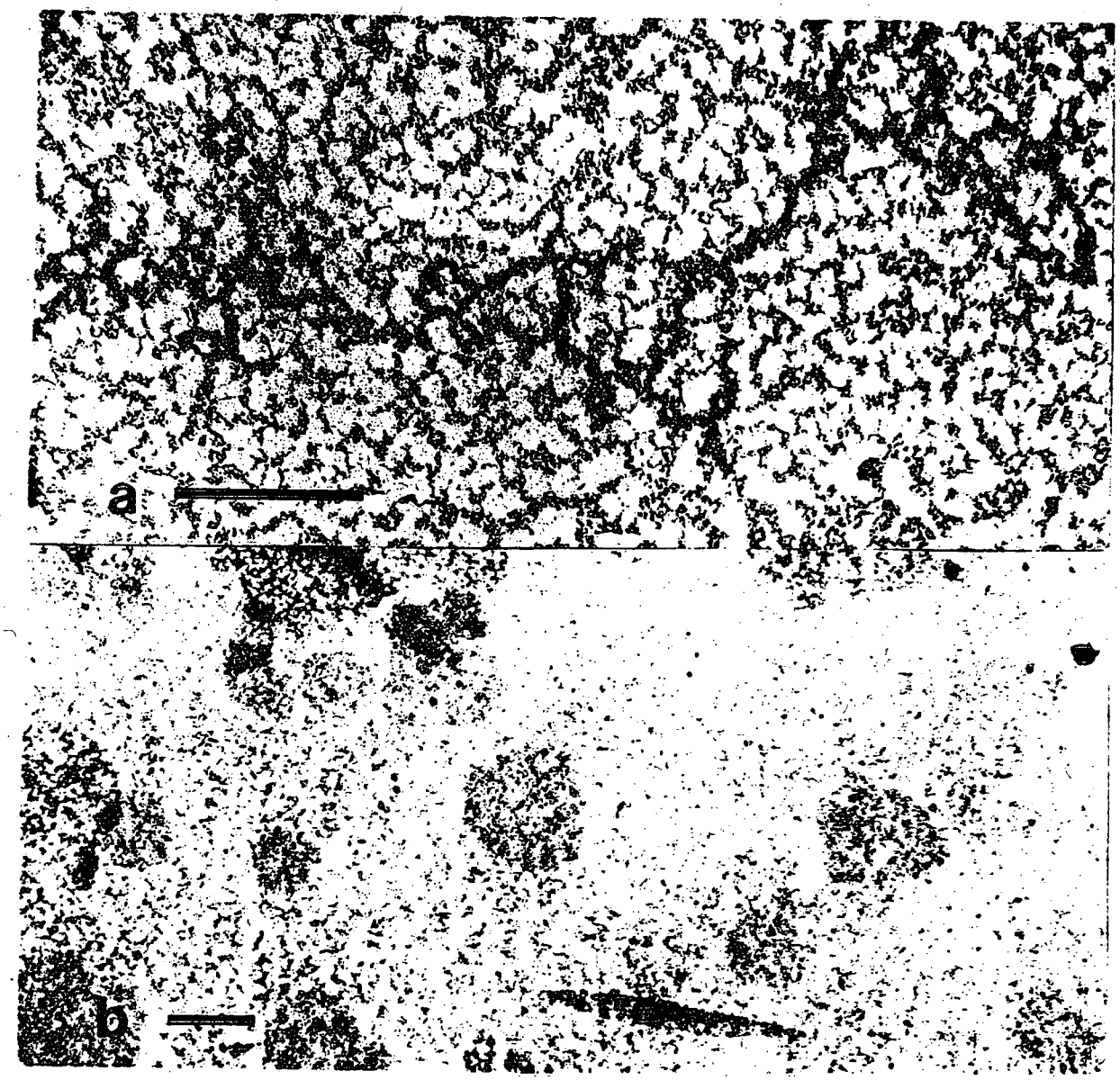
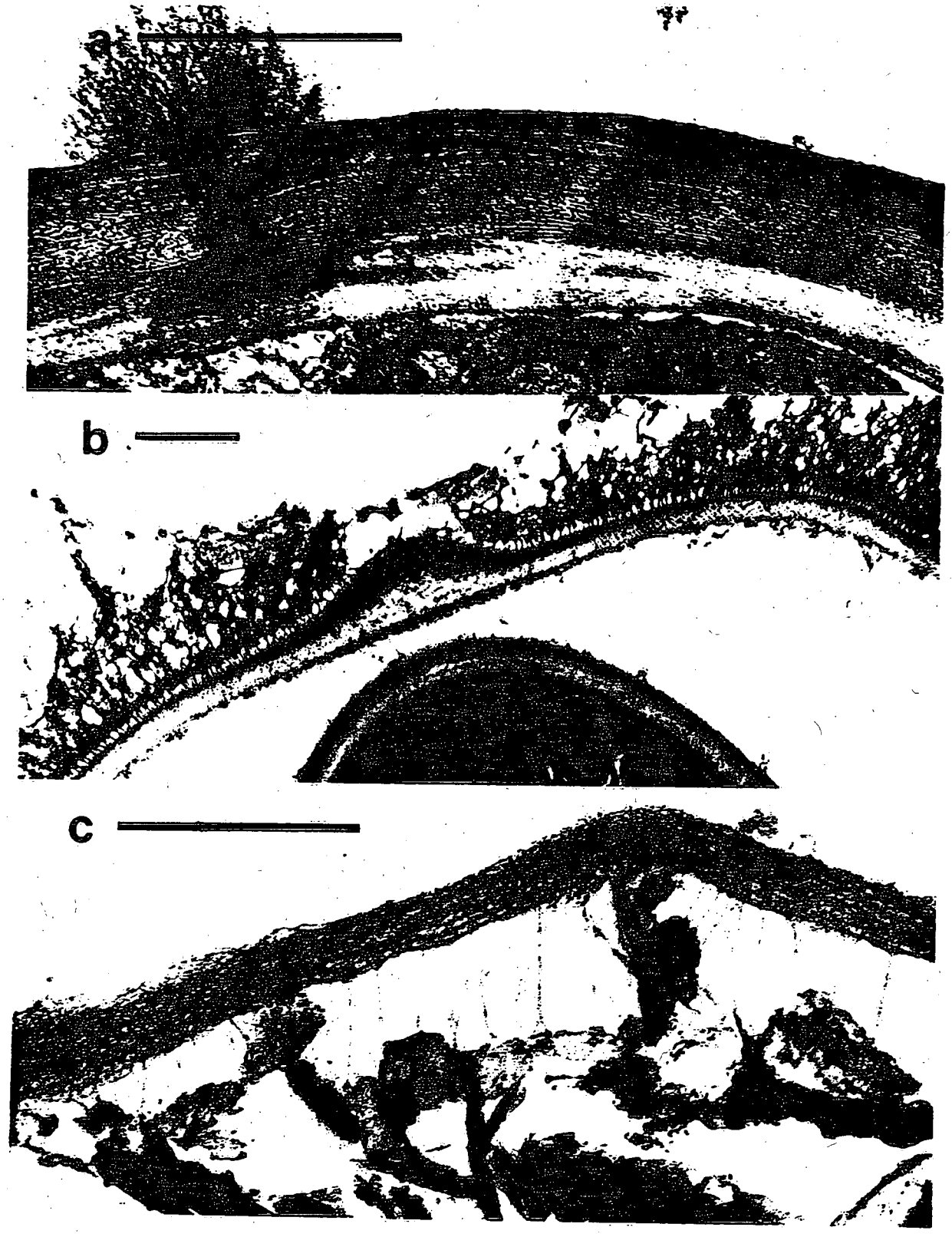


FIG 6 LEPPARD


a
b
c

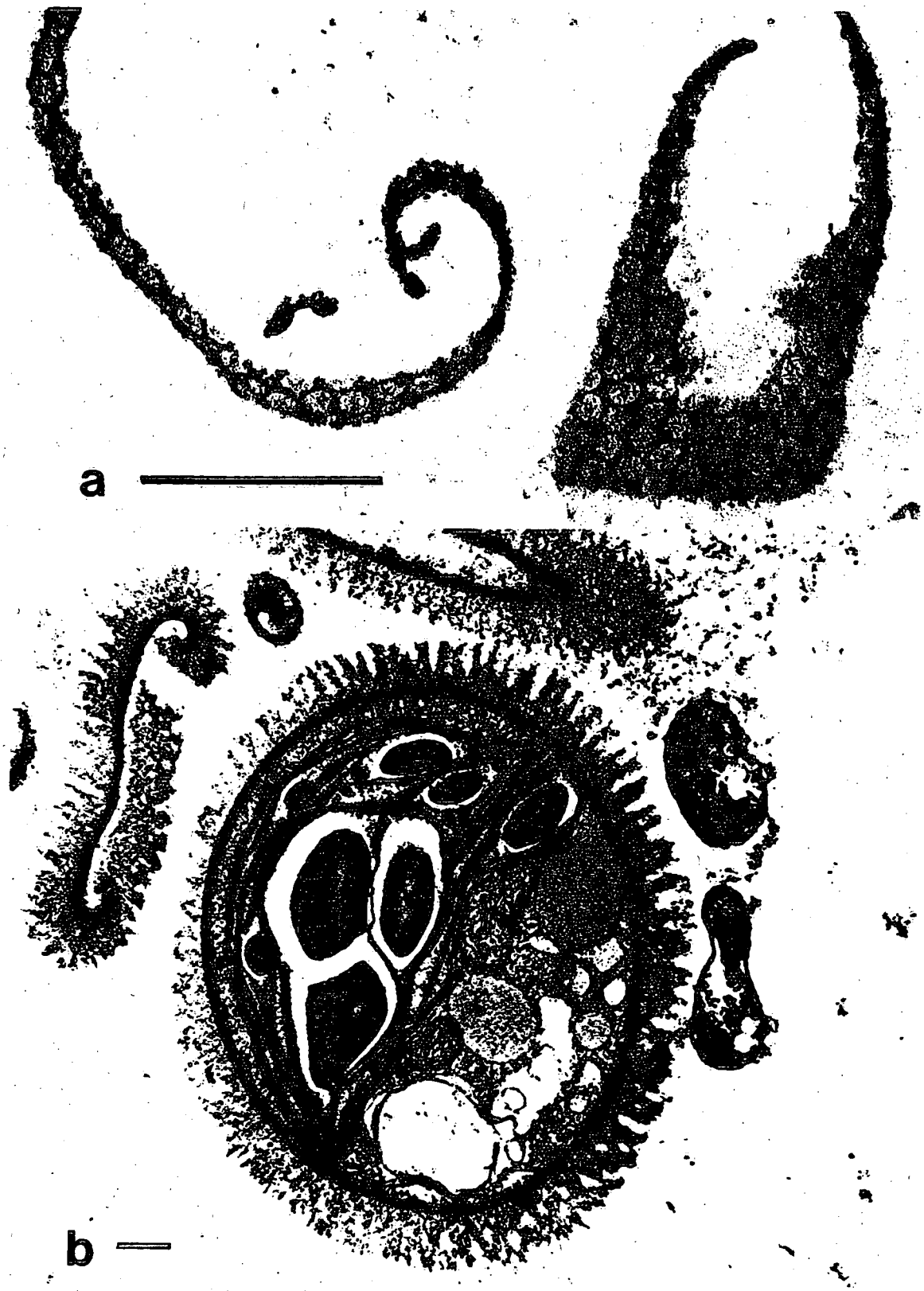


FIG 8 LEPPARI

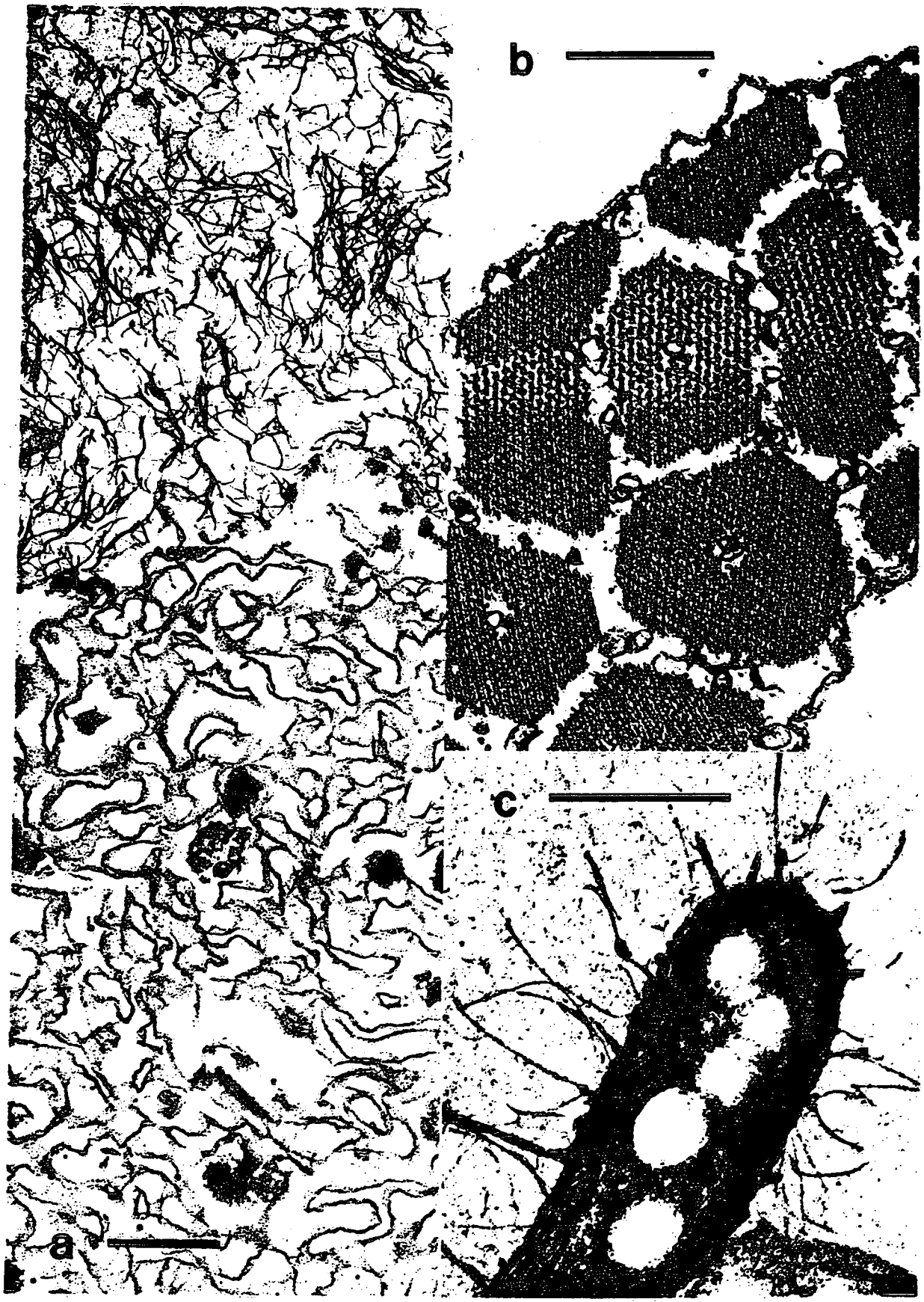


FIG 9 LEPPARD

a
b
c
d

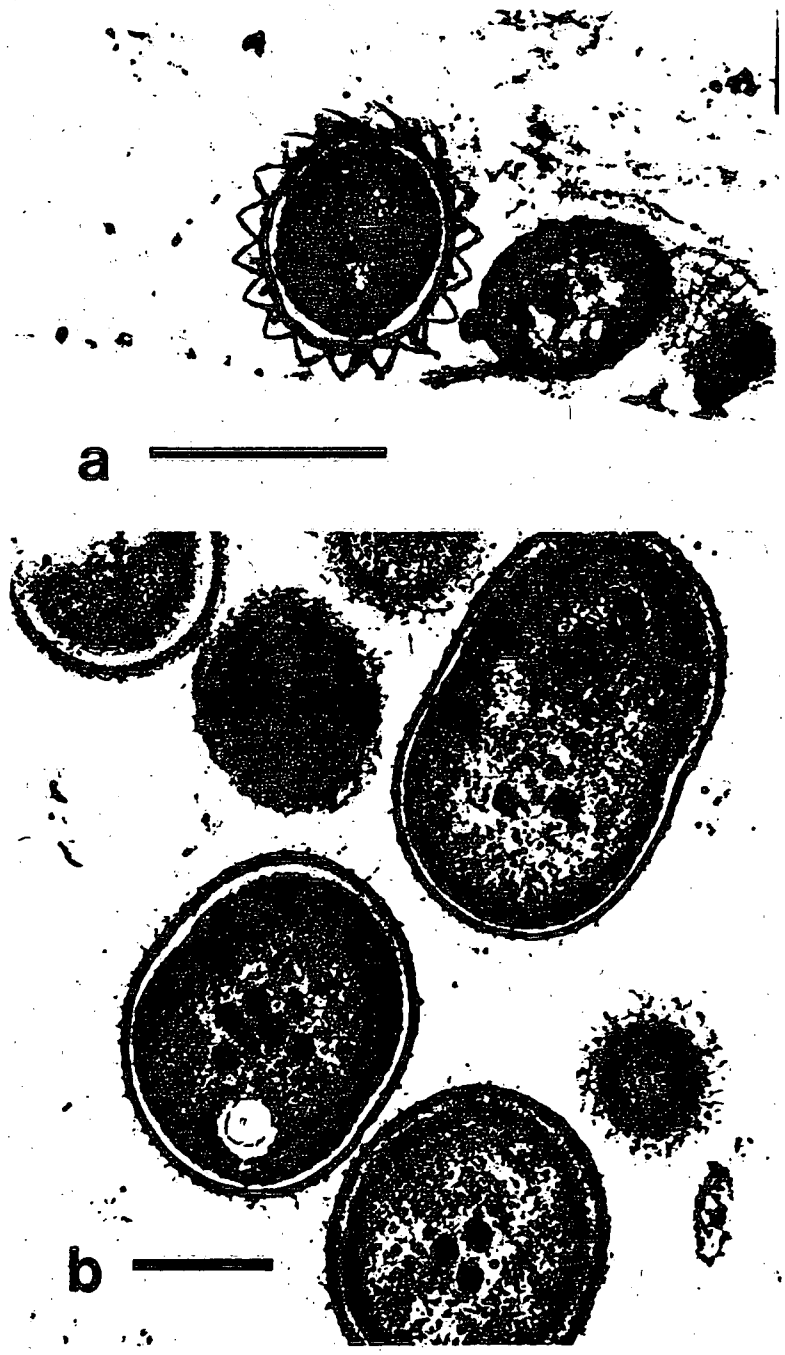
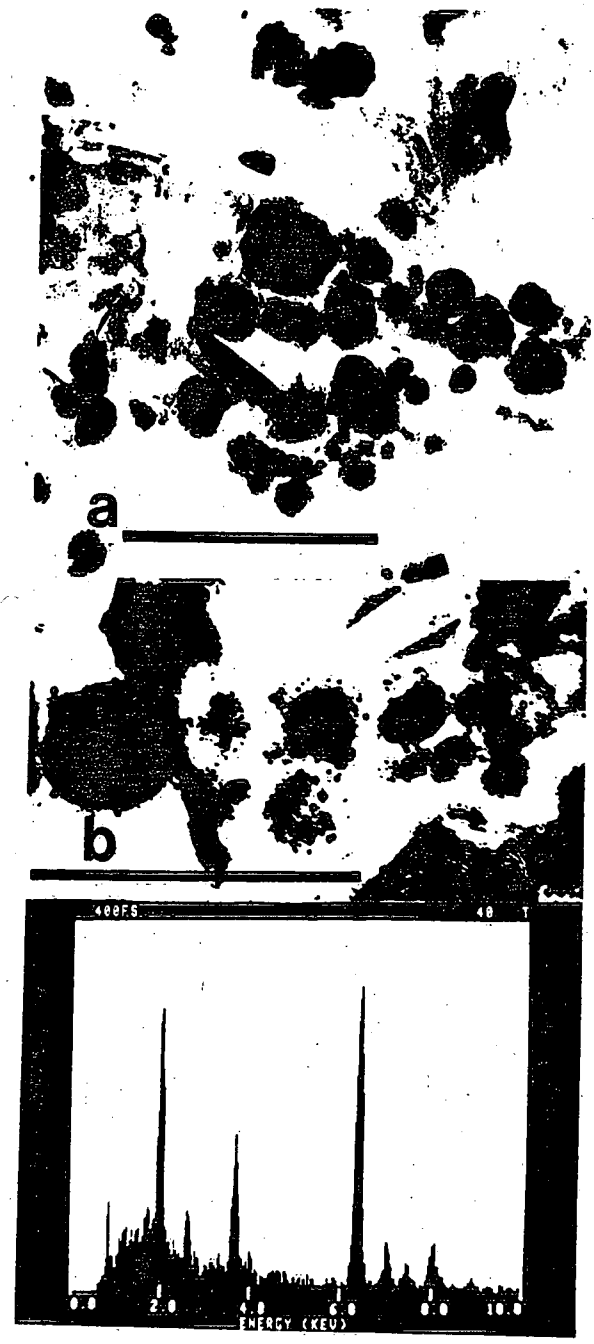


FIG 12 LEPRARI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b
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2.0
4.0
6.0
8.0
10.0
ENERGY (KEV)

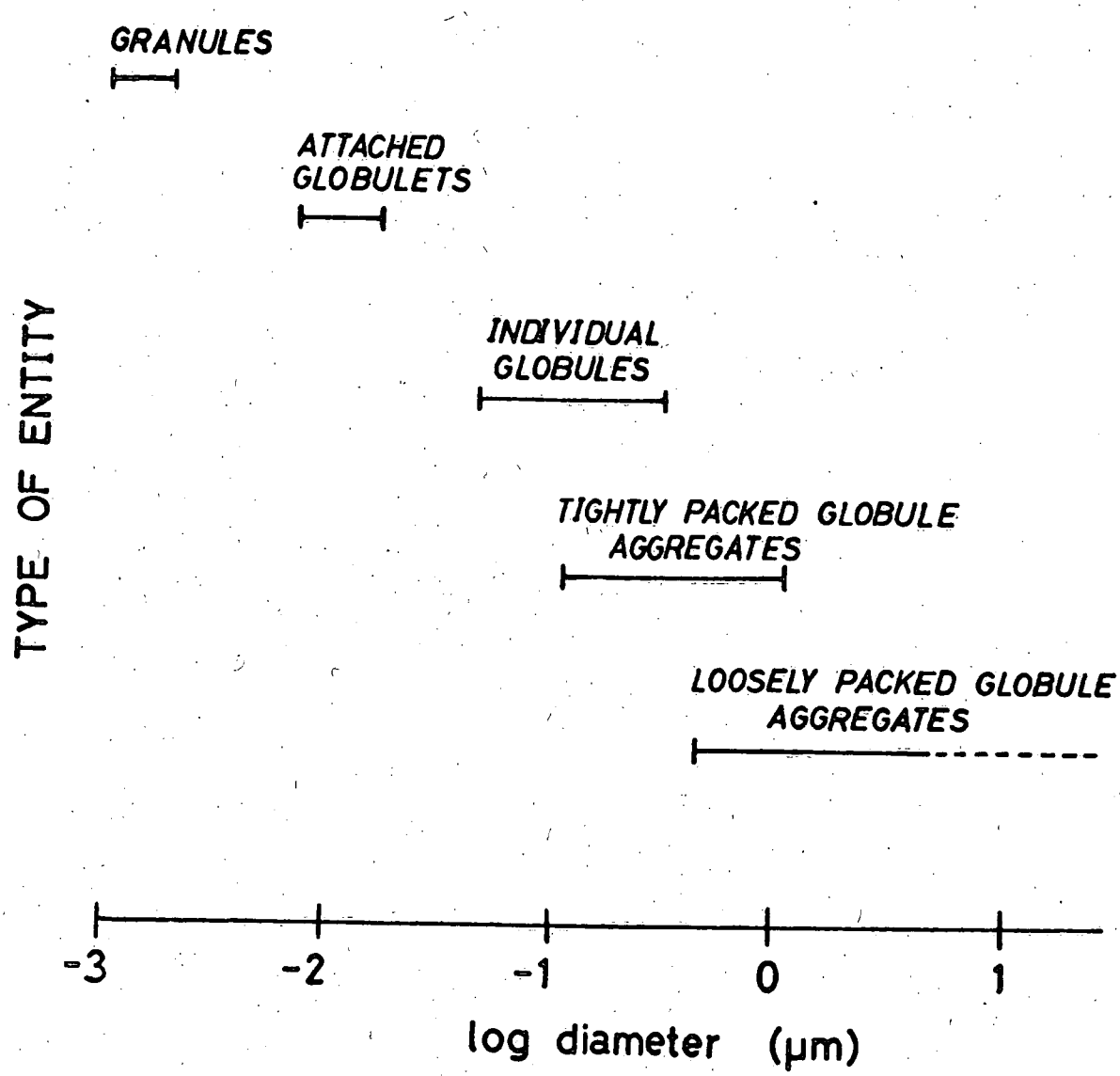
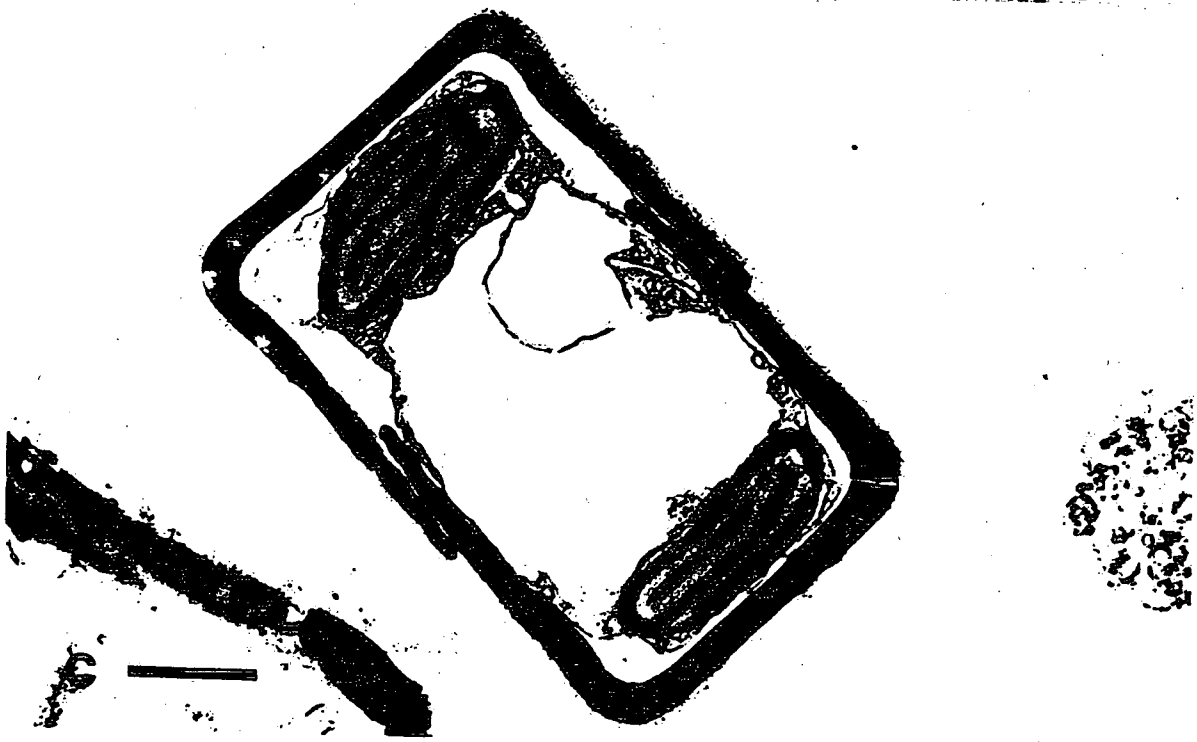


Figure 14. Size classes of iron-rich globules, globule aggregates and globule parts found in an iron-rich lakewater fraction originally isolated on a 0.45 μm filter. This figure was adapted from reference 28.


a


b


c

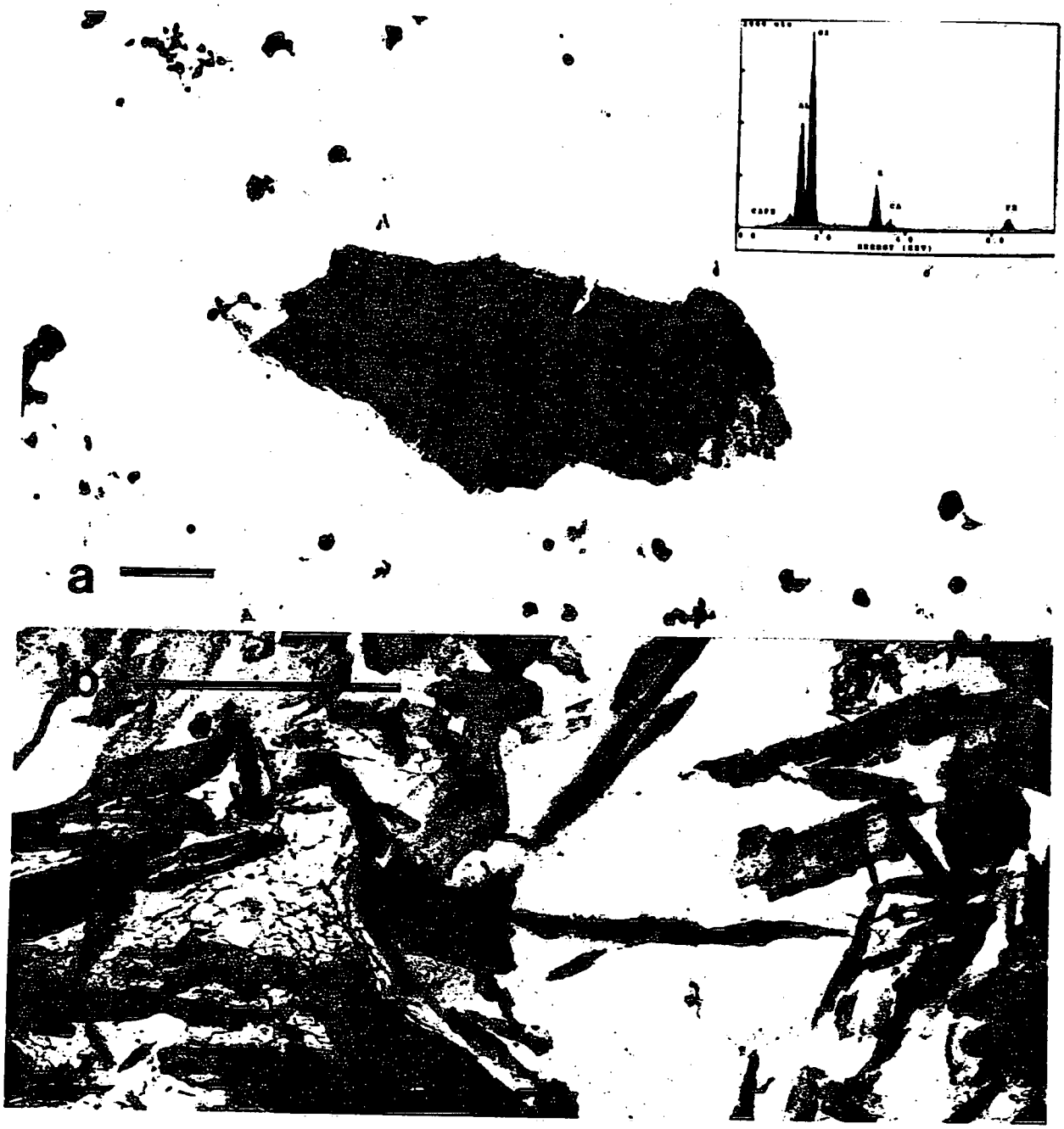


FIG. 16 LEPPARD

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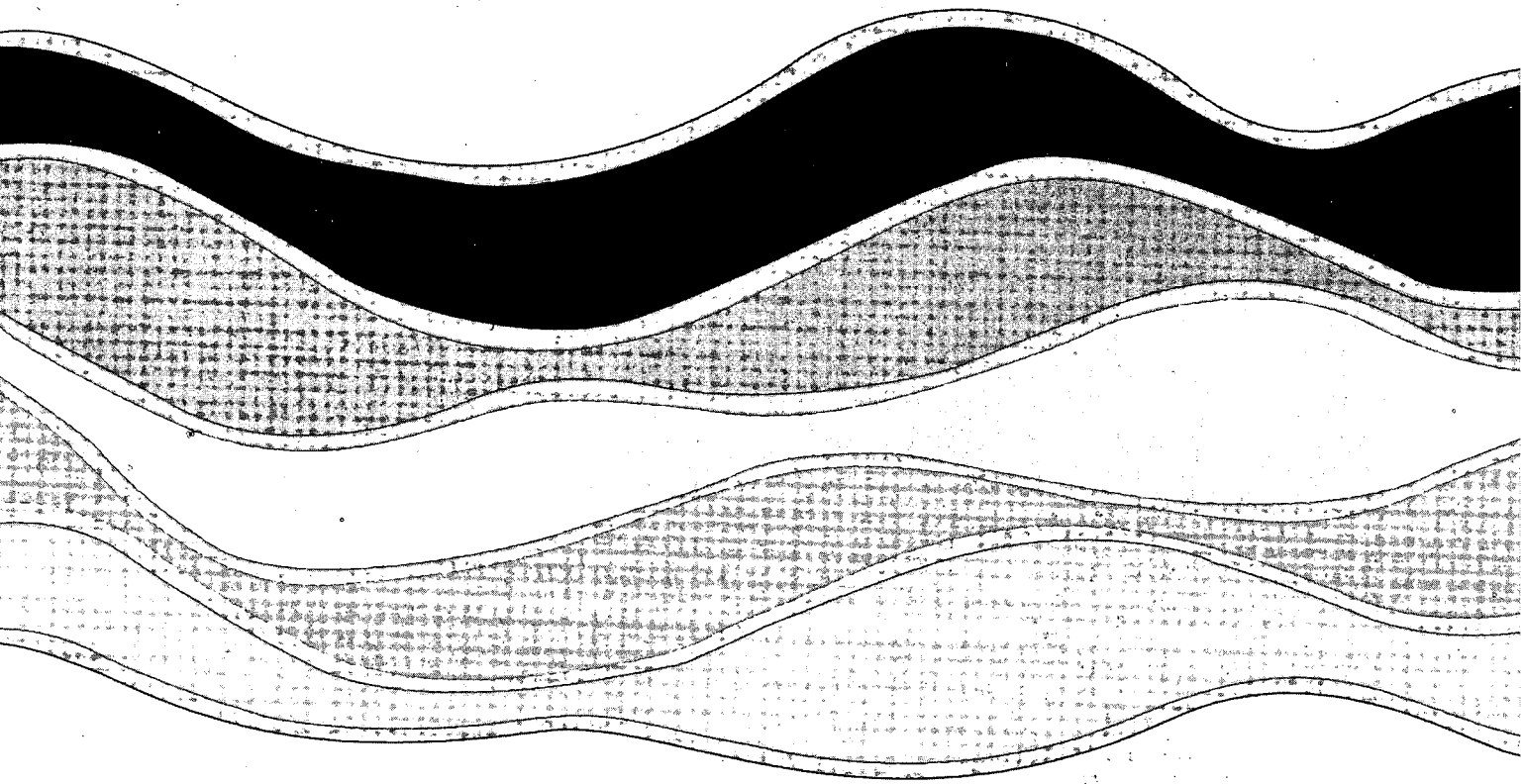

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