DIATOMS

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H

O. F. Cox.
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PRACTICAL DIRECTIONS

FOR

COLLECTING, PRESERVING, TRANSPORTING,

PREPARING AND MOUNTING

DIATOMS.

BY

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PREFACE.

Every person that uses the Microscope for anything except some one special purpose, will probably, at some time or other, be interested in diatoms, from the simple fact that they meet us almost everywhere, and present attractions and beauties which, when once seen, are pretty certain to rivet the attention of the observer. As test objects they have occupied very largely the attention of those who have studied the improvement of the microscope, and that they have exerted a powerful influence in bringing our objectives up to their present high standard, cannot be denied. Unfortunately their life-history has not been so generally studied, because perhaps microscopists have contented themselves with the use of objects obtained ready mounted from the dealers, instead of collecting, preparing and mounting them themselves.

It is to aid them in this that we have brought together the following tracts, which give the latest and best information on the subject. The paper by Dr. Edwards formed a portion of the "Natural History of the State of New Hampshire," of which but a very limited edition was issued. A small portion of the present paper (that devoted to collecting) was printed separately, and pretty widely distributed, but the directions for preparing and mounting have only been accessible in a somewhat expensive form. The papers by Prof. Johnston and Prof. Smith appeared in the "Lens," and are not now procurable, except at considerable cost. We have therefore thought that it would be doing a good service to the cause of microscopy to reproduce these three papers. To the authors we owe our best thanks for their liberal permission to reproduce them.

THE PUBLISHERS.

DIATOMACEÆ.

BY

PROF. A. MEAD EDWARDS, M. D.

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DIATOMACEÆ.

DIRECTIONS FOR COLLECTING, PRESERVING AND TRANSPORTING SPECIMENS OF DIATOMACEÆ.

THE Diatomaceæ constitute a group of organisms of so much interest to the student of natural history, that it is desirable that specimens should be collected in various parts of the world. That such collections may be of value, it is necessary that they should be made in a proper manner; and for the purpose of facilitating the making of such collections, these directions have been drawn up. The directions given should be closely followed, as the methods described have been found, after considerable trial, to be those yielding the most satisfactory results. As the fossil deposits containing the remains of diatomaceæ are most readily recognized, gathered, and forwarded, they will be first described.

Fossil Deposits. Included under this head must be considered the enormous sub-plutonic strata found on the Pacific coast of North America, so that the fossil deposits of diatomaceæ may be said to contain both fresh-water and marine species, though never in a mixed state. In some cases the particular species present indicate the character of the piece of water in which the deposit has accumulated, different forms, or groups of forms, appearing in bays, ponds, lakes, marshes, springs and rivers, and at various points of elevation above the surface of the sea.

The principal fossil deposits of diatomaceæ hitherto discovered contain marine species, and extend over considerable tracts of the earth's surface. The most important stratum of this character is considered to belong to the miocene tertiary, and is found on the Atlantic side of North America, not far from, and, in fact, in some places, reaching down to the coast. It is known to extend from the Patuxent river, in Maryland, as far south as the city of Petersburg, in Virginia. How much beyond these two points it extends has not been ascertained, but is found underlying the cities of Petersburg, Richmond and Fredericksburg, in Virginia, and at many other points in that State, as well as in Maryland. It is desirable to obtain specimens from different points in this bed, as it varies in character, and contained organisms with every few miles of surface, and at different points in its depth.

Strata of this kind vary greatly in appearance, as well as in microscopic character. Therefore the following general directions will suffice to guide collectors in searching for and detecting them.

Gather all earths of light color, varying from a pure white, through different shades of grey, cream, and fawn, to an iron-rust tint. The texture is often friable, and then looks somewhat like clay, especially when it is wet; at other times it is of a hard and stony character, though always more or less porous, and, when soft, of little weight. A moderate magnifying power shows it to be made up of the shells of diatomaceæ. Collect enough to make up three or four pounds' weight, or, say, a block six or eight inches square, and, if possible, from the surface and at various depths, for the reasons already stated. Some of the localities of this material may be mentioned. In Virginia it has been procured in and near Petersburg and Richmond, at Shockhoe Hill and Church Hill, and at Hollis Cliff; and in Maryland, at Lower Marlboro', Nottingham, Piscataway, and Rappahannock Cliff.

Besides the above mentioned, an extremely interesting stratum of a similar character, but in general of harder texture, has been found on the Pacific coast of North America, and extending at least from San Francisco to the lower border of California, if not further, in both directions. This substance makes up the major part of the rocks of the coast range of mountains, and has been named the bituminous shales. It was first detected at Monterey, and is known to microscopists in England as "Monterey stone," but it has since been traced and brought from various points. Santa Cruz, San Pedro and San Diego have yielded excellent specimens, containing many beautiful forms of diatomaceæ. It is usually light fawn-colored, and distinctly stratified. Large fossil shells are found in it; and associated with and in, if not derived from it, is the bitumen of California. At Baldjik, near Varna in Bulgaria, on the Black Sea, is a stratum of stony character, having shells and bones dispersed through it. The diatomaceæ found in it are apparently of brackish-water origin, and this is the only stratum of this kind that is known. But very little of this material has found its way into the hands of naturalists. On the island of Jutland, in Denmark, is found a polishing slate which is rich in diatomaceous forms not found anywhere else. This, also, is rare among naturalists, and a good supply of it is very desirable. At Oran, in Algeria, Africa, and at Ægina and Caltanisetta, in Greece, are deposits containing the remains of diatomaceæ, intermixed with polycystina and foraminifera, and referred to the Cretaceous. In the island of Barbadoes are so-called marls made up of diatomaceæ and polycystina, the latter in great numbers and very beautiful. In the island of Trinidad, at South Naparima, a similar stratum has lately been discovered, which "is considered as connected with the new red sandstone; adjoining to which is the sandstone, probably of the same description, in which the Pitch Lake is situated." At Moron, in Spain, has been found a similar deposit of marine diatomaceæ; and still another was discovered by Dr. C. F. Winslow, at a point about seventy miles south of the town of Payta, in Peru, and about fifteen miles from the Pacific Ocean. Here is a plain separated from the sea by a range of hills several hundred feet high. Within the plain is a depression with nearly perpendicular walls two hundred feet high, the bottom of which depression is at about the level of the sea—perhaps a little lower. The surface of the soil thereabouts is covered with salt. For fifteen feet down there is a deposit containing recent shells, the bones of cetacea, and pebbles; then, for one or two feet, is a yellow loam, and, at the bottom, is the stratum, containing the diatomaceæ, which is from two to four feet thick. The amount Dr. Winslow brought away was very small, and this is all that has got into the hands of microscopists. Prof. Pumpelley brought from near Netanai, in Japan, specimens of a like deposit. Very small fragments of the strata from Jutland, Trinidad, Moron, Payta, and Japan, have been secured; so it is extremely desirable that those localities should be again visited, the geological relations of the strata ascertained, and a plentiful supply of the material gathered. The sub-plutonic deposits seem to be confined to the Pacific coast of the North American continent, and near by. At Five-Mile cañon, near Virginia City, Nevada, is an enormously thick stratum of this character, which is ground and sold considerably under the name of "electro-silicon," as a polishing powder. At Klamath Lake, on the banks of the Columbia and Pitt rivers, and elsewhere, at many points, these deposits have been found.

The rules already given hold good with regard to gathering specimens of all of these deposits. Everything that can be ascertained with regard to their position and relations should be noted. Also, any fossils contained in them, or in the strata above or below them, should be gathered, and their position noted on the labels accompanying them. All specimens should be kept carefully separate (not even permitting them to come in contact) by wrapping each one in paper, placing within a label having written upon it *in ink* the exact locality, date of collection, and name of collector. It is also desirable that note should be made of the depth from the surface at which the specimen was taken, together with any other information that may be deemed of interest, as supposed extent of stratum, slope-upwards towards north, south, east, or west, and thickness.

Lacustrine Sedimentary Deposits. These were called by me at one time sub-peat deposits, from the fact that all I had seen up to that time had been discovered beneath peat; but as the number of these strata which have come into my hands has increased, I have seen many which do not occur under such circumstances; hence the above name has been applied to them as being more appropriate, and indicating their most common mode of occurrence. In England they are called fossil; but in the true acceptation of that term the forms contained in them are not fossils, but are identical with living species.

They are generally of a pulverulent character, and, when dry, are of little weight, so much so as to attract attention. When free from organic matter, as occasionally occurs, they are quite white, looking almost like powdered starch; but most commonly they are grey, which looks dark while the material is wet, but when dried the color is light. A mass of about six or eight pounds' weight should be secured, and the same precautions as to keeping separate and labeling specimens adhered to, as have been already mentioned. As these beds are seldom of any great extent (they often soon become obliterated or covered up), it will be well to secure a good supply of the material while it is accessible. If any shell, wood, or other organic remains should be found dispersed through the deposit, or overlying, or beneath it, they should also be secured, and their position recorded on the label. Likewise, a sample of any superincumbent peat should be kept for future examination. In Sweden and Norway, and in Lapland, these deposits have been used to eke out a scanty supply of flour during bad seasons; but they can hardly be said to be food, for they are not nutritious, but most likely only act by their mass distending the stomach, and thus allaying for a time the pangs of hunger. They have likewise very frequently been employed, under the name of "tripoli," as a polishing material, and are excellent for that purpose. In some parts of this country they go by the name of "marl," but they are not examples of that substance, which is calcareous, being made up of the remains of the shells of mollusca. Specimens from every locality are desirable.

Muds and Deposits from the bottoms of harbors, bays, lakes, ponds, estuaries and rivers. As a general thing these are not of very great value to the microscopist for the remains they contain, and it is only desirable to collect them in localities, or under circumstances where other gathering cannot be made, or when they are known to contain any organisms of great beauty or rarity. The blacker and softer the mud the better, for, if it contains much sand or gravel, the minute organisms will be present in just so much less proportion. As much as can be conveniently transported, say about a handful, should be collected, and, if possible, not dried, but placed in a bottle and tightly corked; or, it may have a little glycerine added to it, which will prevent its drying,—for it has been found that muds, and especially those from salt water, when once dried, are only with difficulty broken down again so as to be cleaned. The mud and slime attached to anchors, buoys, and submerged woodwork, together with the scrapings from the bottoms of vessels containing shells, plants, zoophytes, etc., may be simply dried in the sun, and then have a label attached. The mud from beneath fresh water is of little value, as it rarely contains any organisms of beauty; but the marine forms found in mud are occasionally fine, beautiful and rare.

Guano. This substance often contains species of diatomaceæ not otherwise obtainable. It is the ammoniacal guanos alone, however, which I have found to yield any great number of diatomaceous forms; but there are certain guanos, of which one known as "Bolivian guano" is an example, partly ammoniacal and partly phosphatic, which contain some forms not otherwise obtainable. Quantities of a pound or two in weight should be secured, and the exact locality of the island or other place from which it was obtained, together with the latitude and longitude, and other information that may be collected and deemed of interest, should be marked *in ink* upon the label.

Shell Cleanings. The sand, mud, algæ, zoophytes, and similar matters adherent to marine shells, which are commonly removed by students of conchology, have often been found to yield rich harvests of rare forms of diatomaceæ. Such material can be washed, or, still better, scraped off of the living or dead shells (the dirtier such shells seem the better, of course), placed in paper and plainly labeled with the exact locality, and, if possible, name of the shell and depth of water from which it was taken. Conchologists will do well to save all their shell cleanings for this purpose.

Marine Invertebrata. Specimens of the entire animal, or the contents of the stomachs of echinoidea (sea urchins) and holothuroidea (sea cucumbers), should be secured, as it has been found that many, if not most of them, are vegetable feeders, and thus take into their stomachs algæ which have diatomaceæ growing upon them. The entire animal should be preserved in spirits (if alcohol is not procurable, brandy or whiskey will answer), but if that be not convenient, they, as well as the contents of the stomachs, may be dried without washing in any way. It has been found that holothurians, when they are immersed in spirit, often turn their stomachs inside out, and thus the contents, which are the part most valuable for the microscopic organisms, will be found at the bottom of the containing vessel. When the whole animal is preserved in spirit, the label may be written in ink on stiff paper or parchment, and, when quite dry, tied to the specimen and immersed with it in the spirit. In this way several specimens can be preserved in the same vessel, and space economized. This method will be found to be the best, as labels pasted or gummed on, or otherwise attached to the vessel, are liable to be obliterated from leakage of the contained fluid, or removed during transportation. The stomachs of mollusca (shell fish) and crustaceans (lobsters, crabs) also occasionally yield specimens of diatomaceæ, and it will be well to secure specimens of those creatures in the manner described. The stomachs of fish occasionally contain diatomaceæ, and may be secured.

Soundings. The material brought up from the ocean bed by the sounding-line, or the larger masses procured by means of the dredge, have been found to yield good returns of microscopic treasures when examined. The calcareous shells of foramifera, as well as siliceous polycystina and diatomaceæ, are found in them. When kept for this purpose, note should be made of the latitude and longitude, depth of water, along with the name of the vessel and collector, and the date of collection.

The dust which collects at sea upon the sails or decks of vessels. This kind of material, although not common, has been found to be of interest when examined microscopically. It can generally be scraped up with a piece of paper. When the quantity is so small that it cannot be collected in this way, a piece of damp paper may be laid on it once or twice, in several places, and then folded up before it becomes dry. Latitude and longitude, direction of wind at the time of the falling of the dust, name of vessel, date, and collector's name, should be noted on the label.

Recent gatherings of Diatomaceæ. These are the most valuable, important, and rich of the gatherings containing diatomaceæ on which the student depends for material for investigation, and they are so various in character that it becomes difficult to give general directions that will serve to indicate the modes of procedure to be followed in securing them. To collect diatomaceæ at all thoroughly, a considerable amount of knowledge of their habits is necessary. In general, it may be said that gatherings should be made of marine plants, or algæ, as they are called, which grow entirely submerged beneath the water, attached to rocks, piers, iron or wood-work. The dirtier such plants appear to the naked eye, the richer will be the harvest of minute organisms secured, as the brown coating, seen upon aquatic plants and similar submerged objects, obscuring them, is but a mass of living diatomaceæ. The larger and coarser algæ,—more especially those having a slimy feel,—do not usually yield many diatomaceæ; but the finer brown, red, or green filamentous kinds are commonly covered with them. Detached fragments thrown up upon the beach ought not to be kept if living ones can be found, for they usually have had the diatoms rubbed off from them, and are, besides contaminated with sand. The living algæ taken from their attachment should be dried without washing or much compressing, and may then be placed in layers, each specimen being plainly labeled with the exact locality, date of collection, and collector's name. Fragments of algæe, which may break off from cabinet specimens, and would be rejected by students of the algæ, may yet be of value to the diatomist. Some of the finest collections I have ever seen were derived from this source. When known, the name of the alga should be stated. If possible, it is extremely desirable to secure specimens of diatom-encrusted algæ in spirits. In this way the diatoms will be preserved in almost their natural condition; and those species which are filamentous or grow in chains, will be available in that condition for study.

Fresh water plants clouded with diatomaceæ may be collected and preserved in the same manner as marine algæ. As has been remarked, the finer filamentous species of water plants yield the best results; the marine fucoids, as the "bladder wrack," and similar species, secrete a mucus which seems to be repugnant to the growth of most diatoms; yet upon the stalks of Laminaria, and some other large olive-colored algæ, are found the finer red-tinted species, which are themselves beautiful objects of study, and are, in turn, the homes of hosts of minute forms of life. Water plants, marine or fresh water, should not be cleaned in any way, but merely raised from the water, and, after draining for a short time, be either laid upon a piece of clean paper to dry, or hung up where the air and sun can rapidly evaporate the moisture. Marine plants will usually not dry thoroughly, as the salts present in the water absorb moisture from the air; hence they are liable to mould unless they are packed in paper. The moss-like carpeting seen upon submerged rocks is often made up of beautiful specimens of the filamentous species of diatoms alone, and it will be well to scrape the surface of the stone, and, placing the mass in a bottle, cover it with alcohol, which will become colored from dissolving the coloring matter of the diatoms, and preserve them in the very best manner for future study. Fresh water forms are very often found hanging in green-colored festoons from the exit pipe of drains, sluices, or fountains, and may be preserved in the same way.

The green, brown, or fawn-colored scum, which floats upon the surface of the water of roadside pools, ponds, bogs, marshes, or rivers, consists usually of little else but diatoms, and may be taken up by means of a spoon or bottle, and then preserved in alcohol or dried upon paper. The surface of the sea may be skimmed by means of a net of fine muslin, having an opening left in the bottom, in which a four or six-ounce wide-mouthed phial is tied, and towed at the stern of a vessel. If the sea water be strained through such a net, either by towing behind a boat, or even poured from a pail, the solid matter contained in it will be washed down and gradually collect in the phial, which can then be removed and tightly corked, and another substituted. Some very beautiful forms have been procured in this way. The stain occasionally seen on the surface of the sea in some latitudes, as well as the minute organisms causing the luminosity of the ocean, yield rich crops of diatoms, and should be secured. Such gatherings may be put up as obtained, or have alcohol added to them for better preservation. The collection of aquatic plants from the mouths of rivers is extremely desirable,—such as have been made in the delta of the Ganges yielding interesting results. The refuse of dredging for shells often yields mud, old shells, or algæ; and collectors will do well to secure such. Experience, however, will teach the best places to look for recent diatoms; but the above general directions will prove of service to those who are new to the pursuit, or who collect for others.

It should always be remembered that a knowledge of the exact locality is of the greatest importance,—so that upon the label should be written *in ink* the locality, date of collection, and name of collector. Other facts deemed of interest may also be added.

HOW TO PREPARE SPECIMENS OF DIATOMACEÆ FOR EXAMINATION AND STUDY BY MEANS OF THE MICROSCOPE.

Having accumulated a number of gatherings of rough material, which, a cursory examination has shown, contain specimens of diatomaceæ, and which, it is judged, it will answer to clean and otherwise arrange and put up, or as it is technically termed, "mount," for future study, the intending diatomist requires to be informed how he may best set about preparing his specimens in the most advantageous manner. The author of the present sketch has published, in the seventh volume of the *Proceedings of the Boston* (Mass.) *Society of Natural History*, certain directions for collecting, preparing and mounting diatomaceæ for the microscope; and as that paper contains a large part of the information he desires to impart at the present time, he will draw upon it pretty freely, supplementing it to such a degree as later investigations warrant, or as may seem desirable.

Although most of the published treatises on the use of the microscope in general profess to give directions for mounting objects in such a manner as to preserve them for almost any length of time, and at the same time exhibit their characters to the best advantage, and although we have in the English language at least three books treating specially of this subject of the preparation of microscopic objects, yet hardly any one of these volumes gives any concise, practical, and at the same time, reliable descriptions of the best methods of collecting, preparing and mounting specimens of diatomaceæ. In books, generally, when the preparation of these organisms is treated of, it is usually the fossil deposits which are considered, and even such directions as relate to these are for the most part meagre and unsatisfactory; and, when the specific and special directions are, as is often the case, copied from one book into the other without having been tested by the copyist, any faults they may have possessed, as originally written, are merely repeated and not eliminated. To prepare and mount specimens of diatomaceæ, for the purpose of sale alone, is one thing, and to prepare and mount them, so as to preserve and exhibit their natural characters and fit them as objects of scientific study, is another and very different thing. The latter can only be attained after considerable practice, and to do it properly a considerable amount of knowledge of their natural history is plainly necessary.

The diatomaceæ should always be prepared and put up for a special purpose,—that of exhibiting characters peculiar to genera and species; and to do this those characters must of course be known. Muds, guanos, dredgings, and gatherings of that description, can seldom be used for the purpose of exhibiting such characters, and when they can, in exceptional cases, be so employed, it is when the forms they contain are selected out in the manner to be described hereafter. Gatherings, likewise, which contain many species in a mixed condition, should, as a general thing, be rejected unless there be present something of special importance, such as rare species, or some large and fine or distorted forms of common species. But even in such cases it will be found best not to mount the gatherings as collected, but to select out the forms desired and place them upon slides by themselves, and in such media as will exhibit their peculiarities to the best advantage. Of course it may be desirable to study the geographical distribution of the diatomaceæ; and then mixed gatherings become of value as exhibiting the number of forms occurring at a particular station. Then, again, the fossil as well as the semi-fossil deposits and guanos may be cleaned and mounted as obtained; but even then it may become desirable, if space can be spared in the cabinet, to have the various species found in each gathering separately mounted, so that they may be at any time studied in comparison with similar forms from other localities.

General directions for collecting diatomaceæ have been already given in the preceding chapter; but it will be desirable to again allude to a few points in connection with this portion of our subject. Some years since, an article entitled "Hunting for Diatoms" was published in a London journal called *The Intellectual Observer.* The author's name was not given, but internal evidence would seem to indicate that it was penned by a deceased botanist of note, who was a decided authority on this branch of biology. This paper contains some valuable hints respecting the places in which to look for diatoms, and some of the suggestions contained therein I have ventured to transfer to these pages, as they will be found of value to the intending diatomist. Thus, the exquisite *Arachnoidiscus, Triceratium Wilkesii*, and *Aulocodiscus Oregonensis*, may be looked for on logs of wood which have been floating in the sea, and imported from New Zealand or Vancouver's Island. So, on logs from Mexico and Honduras may be found the curious *Terpsinœ musica.* The nets of fishermen, especially from deep water, may yield algæ bearing such forms as *Rhabdonema arcuatum or Adriatum*, *Grammatophora serpentina* and *marina*, various *Synedras*, and other fine forms. On oyster shells may be found algæ bearing upon their fronds *Biddulphia regina, Baileyii* or *aurita. Rhizosolenia styliformis* is said to be almost sure to be there likewise. After a ship is unloaded, and as it floats higher in the water, its sides may be searched for treasures of the diatom world, and *Achnanthes longipes* and *brevipes* found, or even *Diatoma hyalinum* and *Hyalosira delicatula.* The sea grass, or *Zostera marina*, growing along our coast, often bears upon its waving ribbons fine forms of diatoms, and that used for stuffing chairs, and lounges or mattresses, and imported from abroad, will yield foreign species to the collector. There is a plant known in England as "Dutch rushes," which is imported into that country from Holland, and which is used for chair bottoms. These plants grow in the brackish water of the marshes, and hence upon them are to be found the delicate *Coscinodiscus subtilis*, *Eupodiscus argus*, and *Triceratium favus*. Both of these two last named forms occur commonly on our Atlantic coast, and muds from Charleston, S. C., and Wilmington, Ga., have provided me with them in plenty. Cargoes of bones, which present green incrustations from having lain in the water for some time, are said to yield diatoms, some of which may be rare, as coming from foreign ports. The State of New Hampshire has not yet been sufficiently gone over for it to be said what the characteristic forms of diatomaceæ growing within its boundaries are, but yet we may safely predict that the lakes, ponds, streams, and sea-coast of that State will yield to the searcher ample material of beautiful forms.

If the microscopist wishes to mount a few slides of recent diatoms, just to show what diatoms are, nothing is easier. It is only necessary to boil a small mass of them in strong nitric acid in a test tube over a spirit lamp, and, when the acid has ceased to emit red or yellowish fumes, wash them thoroughly with clean water, allowing them to settle completely. Then a little of the clean sediment, consisting almost entirely of the shells of the diatoms, is taken up by means of a "dip-tube," and placed upon the central portion of a glass slide. Here it is dried, and the slide warmed over a lamp; then a drop of Canada balsam is permitted to fall upon the diatoms. As soon as all bubbles have cleared off from the balsam, a warm cover of thin glass is carefully laid upon it and permitted to settle into place. When cool, it is ready for examination by means of the microscope, any balsam which has exuded around the cover being washed off with alcohol. In this way rough and tolerably clean specimens may be obtained; but such would not, or, at all events, should not, satisfy the student of the diatomaceæ. For him more elaborate methods are necessary, and these we will now proceed to consider.

Apparatus and Chemicals necessary. A chemist's retort-stand, which is a heavy iron plate with an upright rod projecting from one side of it. Running on this rod, and so arranged that they may be fixed by set-screws at any height, are a series of rings of various diameters, which are to be used to hold the vessels in which the specimens are to be manipulated over the source of heat used. Mr. C. G. Bush, late of Boston, Mass., who has had considerable experience in cleaning diatomaceæ, tells me that he uses a lamp burning petroleum oil, as cheaper than a spirit lamp, and, to support the vessels he employs, has a little metal arrangement on the top of the chimney, such as is supplied for the purpose of holding a small tea-kettle and the like. The only objection to the oil lamp is, that, unless the wick be well turned down, we are liable to have our vessels blackened. However, the heat given off by burning petroleum is very great, and I have often used such a lamp with advantage. If desired, of course, the source of heat may be gas, burned in a Bunsen's burner, or a spirit-lamp; and this last, especially if it be supplied with a metal chimney to cut off draughts, is, all things considered, the best, as it is very cleanly, not being liable to smoke the bottom of the glass or porcelain vessels used. If we are going to work with large quantities of material, we shall require a small sand-bath to heat the glass vessels upon. In small quantities, the diatoms may be boiled in test-tubes, when some sort of holder will be required. The metal ones, sold by dealers in chemists' apparatus, are extremely handy; but I have found that we can make very good ones out of old paper collars. One of the kind called "cloth-lined" may be cut into strips about three-quarters of an inch wide and three inches long. Such a strip is folded around the test tube, near the top, and the ends, brought together, are held between the fore-finger and thumb. In this way the tube is firmly grasped, and can be held over the lamp without much danger of burning the hand, as the paper collar strip is a bad conductor of heat; or the paper strip may be grasped in an "American clothes-peg," which has a spring to force its parts together. Large quantities of diatoms are best boiled in porcelain evaporating-dishes, glass flasks, or beaker-glasses. The last-mentioned vessels are also by far the best things for washing them in. A few, say three or four, glass stirring-rods will be found useful; and one or two American clothes-pegs to take hold of hot evaporating-dishes with. Then there will be required a few dip-tubes, made of small glass tube, drawn out over a flame, so that the opening is considerably diminished. The mode of making these cannot be given here, but will be found in books on chemical manipulation; and it will be well for the student to learn to make his own dip-tubes, as a number will be required first and last, and they are easily broken. Of course there will be required a number of glass slides, of the usual dimensions of three inches by one. These should be of as white glass as possible, and it will be found best to procure those with ground edges, as they are the neatest in appearance. Only such as are free from scratches or other blemishes in the central square inch should be used; and, although even such as have bubbles or scratches near the ends only will not look ornamental in a cabinet, we should remember that microscopic objects are not generally mounted to look well in a cabinet, but to be useful out of it; so that if the central and useful portion of the slide be perfect it need not be rejected. Some persons make their own glass slides, but I have never found it answer to do so, as it is difficult to get the right kind of glass, not at all easy to cut it or grind the edges, and it is liable to be scratched while cutting or grinding. Thin glass, such as is made on purpose for microscopic use, will be required; and this, also, it will be found best to buy ready cut rather than attempt to cut it for oneself. The thin glass used for covers may be of different thicknesses, but the thickest made will not do for diatoms, and a certain amount of the very thinnest will be required for small and delicately marked forms, on which very high power objectives will have to be used. The covers must be perfectly clean, which may be insured by soaking in caustic potassa solution, and then washing thoroughly in clean water. The thinner kinds of glass are rather difficult to clean; but with a little extra caution it may be accomplished, the last polish being given to it by a piece of an old and well-worn cambric handkerchief. The covers, always round, should be separated into sizes and thicknesses, so that the exact kind of cover required can be found without having to search for it by turning over a number, scratching or breaking them, and losing much valuable time. We shall also require a pair of forceps for holding the slides over the lamp; and such as are sold at house-furnishing stores and by grocers, under the name of American clothes-pegs, and which have been already mentioned, are by far the best I have ever seen or heard of. A small pair of brass forceps which close with a spring will be needed, and they are best set in a wooden handle, so as to protect the fingers from the heat; and another pair, which spring open and may be closed by means of the finger and thumb, will be wanted for taking hold of and adjusting the thin covers. I do not advocate the use of paper covers for slides, but labels of some kind will, of course, be required, and I have found the plain circular white ones to look the best. There are very pretty square labels sold by dealers in these things that I have used and liked. For making cells to hold specimens put up in fluid, a turn-table and brushes and some cement will be necessary. The cement I use and prefer above all others is good old gold size, used warm.

The chemicals required are nitric acid, sulphuric acid, hydrochloric acid, bichromate of potash, caustic potash, alcohol, and, above all, a plentiful supply of clean, *filtered* water. The water should be such as leaves hardly any residuum when a quart of it is evaporated to dryness; and it must be filtered just before use, to remove any minute organisms, diatoms especially, which it may contain. A certain amount of washing soda will be wanted, if guanos are to be cleaned.

We will now proceed to consider the manipulations necessary to prepare the various kinds of gatherings, always remembering that these methods will have to be modified to a certain extent for each specimen.

Recent Gatherings. If there be sand in the gathering, it will be well to remove it before using acid, by shaking it in clean water and pouring off before the diatoms, which are lighter than the sand, settle. The water holding the diatoms in suspension may be poured into a test-tube or beaker, the diatoms allowed to settle, and as much of the water poured off as possible. The diatoms are now covered with nitric acid to about the height of half an inch, and allowed to stand for a few minutes. Usually, some chemical action takes place, and it will be well to wait until it subsides. The test-tube or beaker is then held over the lamp and carefully heated until the reaction of the acid upon the organic matter of the diatoms ceases. Thereafter, and while the liquid is still hot, I have found it often advantageous to drop in one or two fragments of bichromate of potash. The organic matter is more thoroughly destroyed in this way than when the acid is used alone. Thereafter it is well to pour the acid and diatoms into a capacious beaker of clean water, washing the tube or smaller beaker out with a little water, and adding this to the other. After the diatoms have all settled, which will often require hours, the supernatant fluid is carefully poured off, and a fresh supply added; and this must be repeated several times until all of the acid and colored chromium compound has been removed. When this point is arrived at can only be ascertained from experience. In this way the valves and connecting membranes of the diatoms are usually separated and cleaned ready for mounting, which process will be described hereafter.

Muds will have to be treated in a somewhat different manner from recent gatherings. If the mud is dry, it will have to be broken down by boiling for a few minutes in a solution of caustic potassa, the strength of which must be apportioned to the particular specimen under treatment. After it has been broken down into a soft mud, all of the potash is thoroughly washed off by means of clean water, and replaced by nitric acid, as in the case of recent gatherings. This is boiled, and a little bichromate of potash added as before, and the whole washed. It very seldom happens that the diatoms occurring in mud will be sufficiently cleaned by this process, so that it has to be supplemented by another. The sediment is therefore washed into one of the evaporating dishes and allowed to settle, and as much of the water poured off as possible. Then sulphuric acid, in quantity to a little more than cover them, is poured in, and the vessel gradually and carefully heated. As soon as the liquid shows signs of boiling, bichromate of potash is added, a very little at a time, until the green color first formed by its reaction upon the organic mattter begins to assume a yellowish tint, when no more is dropped in; but a few drops of hydrochloric acid are permitted to fall in, and the liquid is allowed to cool. Of course it will be best if the person undertaking to clean diatoms is somewhat versed in the use of chemcals; but at any rate care must be taken not to drop any of the acids upon the clothes or skin, and great caution must be exercised in not inhaling any of the vapors given off. Those evolved after the addition of the hydrochloric acid are especially irritating and dangerous, and must be avoided. As soon as the liquid has cooled a little, water should be added cautiously, as great heat will be generated thereby, and there will be danger of its boiling over. Thereafter it may be poured into a large beaker-glass of water and thoroughly washed, as in the former case. If it be found that the precipitate is not quite white, it will be necessary to boil it again in sulphuric acid, with bichromate of potash and hydrochloric acid, until it is quite clean. If, on examination by means of the microscope, it is found that there is much flocculent matter present besides the diatoms and sand, this can be removed by boiling for a few seconds in a weak solution of caustic potash, and washing quickly and thoroughly with plenty of clean water. When we have recent gatherings of filamentous or stipitate forms of diatomaceæ, which we desire to preserve in the natural condition, they should be immersed for about twenty-four hours in alcohol to dissolve out the endochrome. If this does not answer, it will be well to soak the mass of diatoms or plants upon which they are adherent in a solution of hypochlorite of soda, an impure variety of which is sold in the shops under the name of Labarraque's disinfectant, for about the same length of time. This will generally destroy all color, and leave the specimens transparent. It is best, however, in many cases not to remove the endochrome, but leave it, and mount the specimens in such a way as to show them in as natural a condition as possible. How this may be done will be described hereafter.

Guanos. The preparation of these substances so as to obtain the microscopic organisms they may contain is rather difficult, tedious, and dirty, and should only be undertaken by a person somewhat versed in chemical manipulations, and in a proper room as a laboratory, where there is no danger of harm resulting from the fumes evolved. As the ammoniacal guanos are those which contain the most diatoms, and consequently which answer best to clean, we will begin with them, and take as a type that which comes from the islands on the coast of Peru. As it comes into commerce this guano is a moist powder of a light iron-rust color, smelling strongly of ammonia, and having scattered throughout its mass lumps of ammoniacal salts of a more or less solid consistency. The guano should be thinly spread out upon a stiff piece of paper and exposed to the air, and, preferably, to a moderate heat for several days or even weeks. In this way most of the moisture and much of the ammonia will evaporate, and less acid will be required to clean the guano. It will now have become much lighter in color, and crumble to a dry powder. A tin pan is now about half filled with a solution of common washing soda in clean filtered water, and placed over some source of heat, as on a stove. The strength of this solution is not a matter of any great moment, and must vary with the guano manipulated. As soon as it begins to boil, the guano is dropped gradually in, a little at a time, while the liquid is stirred with a glass rod or stick of wood. Considerable effervescence takes place, ammonia being given off, and therefore it must be kept continually stirred, and care exercised to prevent its boiling over. After a while it is poured into a plentiful supply of clean water, and washed therewith several times, care being taken to permit all of the diatoms to settle. As soon as the wash-water is only slightly colored, the guano is transferred to a good sized evaporating dish, and covered with nitric acid, and boiled. While it is boiling, a few crystals of bichromate of potash are dropped in, and the material washed as in the case of muds. Thereafter the diatoms are boiled in sulphuric acid with bichromate of potash and hydrochloric acid, as before described.

Phosphatic guanos, as that from Brazil, are somewhat more difficult to treat. They are generally drier than the ammoniacal kind, and must be boiled in a large quantity of hydrochloric acid as many as three times, and the acid must be poured off while still hot. Thereafter nitric acid and sulphuric acid and bichromate of potash must be employed, as in the other case.

Lacustrine Sedimentary Deposits. For the most part these are pulverulent, and easy to clean. Some, as found in nature, are so pure that they require no cleaning except washing in clean water. Burning on a plate of platinum or mica will often serve to clean some specimens, but it will, in general, be found best to boil in nitric acid with a little bichromate of potash, and subsequently in sulphuric acid and bichromate of potash, with the after addition of hydrochloric acid. Occasionally a certain amount of flocculent matter will be left, which it will be necessary to remove with very careful heating, not boiling, in a weak solution of caustic potash, and immediately pouring into a large quantity of clean water and thoroughly washing.

Marine Fossil and Sub-Plutonic Deposits, being stony and possessed of very much the same physical characters, are manipulated in the same manner. A small lump of the deposit is placed in a test-tube, and covered with a strong solution of caustic potash. It is then boiled for a few minutes, and usually it immediately begins to break up and fall down in the shape of a soft mud-like material. At once the liquid, with the suspended fine powder, is poured off into a large quantity of clean hot water, and if the whole of the lump has not broken down into a powder, what remains has a little water poured over it in the test-tube, and it is again boiled. It will be found that a little more will now crumble off. This is added to the rest in the large vessel, and if the lump has not now broken down, it is again boiled in the alkaline solution and in water alternately, until it has all been disintegrated. It is then all permitted to settle for at least three hours, when it is thoroughly washed and boiled in hydrochloric acid for about half an hour. There is then added an equal amount of nitric acid, and the boiling continued for a short time. It is then washed and heated in sulphuric acid, with the addition of bichromate of potash and hydrochloric acid.

All mixed gatherings of diatomaceæ, and particularly all muds and deposits, should be separated into densities, so that for the most part the larger forms are collected together, free from sand, and separate from the smaller species and broken specimens. This is done by using a number of beaker glasses, of various sizes, in the following manner: Into a one-ounce beaker the cleaned diatoms are placed, and the vessel filled with water. It is then well stirred up by means of a glass rod, and, after resting about five seconds, poured off carefully into a six-ounce vessel, so as not to disturb the sand which has settled.

Again the vessel is filled up with water, stirred, allowed to settle for the same length of time, and poured into the same vessel. This is repeated until it has been done at least six times, when we shall find all of the sand, free from diatoms, in the small beaker. This can be thrown away, and as soon as the material in the large beaker has settled, it is returned to the small one, and the same process gone through with, only extending the time of settling now to about ten seconds. The next density is that which settles in twenty seconds; and on on, five or six densities may be obtained, and if carefully prepared they will be found to contain forms varying very much one from the other. The large species of *Triceratium*, *Aulacodiscus*, and the like, will be found in the coarsest density, and the broken diatoms in the lightest.

Preserving and mounting specimens so as to have them in a condition for study at any future time. Of course, when possible, diatomaceæ should be studied in the living condition. But there are many forms which have not been as yet found living, and these can only be studied as dead skeletons; and, in fact, it is in the dead skeletons of the diatomaceæ that many of the most marked characteristics are to be found; and on such characteristics species have been founded. Besides, the most beautiful sculpturing of the valves is only to be seen after everything has been removed but the siliceous cell wall I have termed the skeleton. Therefore I advocate the cleaning of a portion, at least, of every gathering in the manner described, so that nothing will be left but the clean siliceous cell-wall.

If we desire to keep specimens in a state as near that they present when living as possible, we have to put them up in some preservative fluid in which they will not decay, and in which the softer parts will be preserved. Unfortunately these soft parts do not keep well; but the fluid which I have found to be the best for the purpose is distilled water, which has to every fluid ounce two or three drops of wood creosote added, and thereafter a sufficient number of drops of alcohol, which will be about double the number of the drops of creosote, to make the creosote soluble in the water, which it is only to a very slight degree under ordinary conditions. I do not advocate any fluid containing glycerine, or, in fact, any of the preservative fluids described in the books treating of the preparation of microscopic objects. The vessel in which the fresh specimens of diatomaceæ are put up are what are known to microscopists as "cells," but how these are made cannot be gone into here, as the description would occupy too much space and time. Suffice it to say that I prefer cells made of old japan gold size, which can be procured of dealers in microscopic materials. Within such a cell, of sufficient depth and immersed in the preservative fluid, a few of the diatoms, or a scrap of the plant upon which they are growing, is placed, and the glass cover fixed over it in the manner described in the books upon manipulation. The filamentous forms are thus preserved almost in their natural condition; but, on account of the presence of the endochrome, the sculpturing of the siliceous cell-wall is almost invisible. To show this character, while the filamentous form is preserved, another method of mounting is employed. A thin, clean covering glass is selected, and laid upon a clean piece of paper. A large drop of distilled water is then allowed to fall upon it, and in this drop the filamentous diatom is thinly spread out. Then the cover is taken up by means of a pair of forceps, and held over the flame of a spirit lamp, which has been turned down so as to be quite small and steady. The cover is held some distance above the flame, and judiciously manipulated, so that the heat is evenly distributed over it, and it does not crack. As soon as all the water has been driven off without the formation of bubbles, the glass is brought gradually down almost in contact with the flame, and held at that point for a few minutes. Then the diatoms will be seen to turn black, on account of the charring of the organic matter contained in them. After a while this black carbonaceous matter will burn off, and they will become quite white. If, however, there seems to be any difficulty in burning off the last portions of carbon, the cover is lowered once or twice to come in contact with the top of the flame, and then raised again. In this way it will become red hot for a moment; and everything will be burned off except the siliceous portions of the diatoms. Now the cover is removed slowly from over the flame, and held in the forceps until it is cold, but by no means laid down upon any surface until it is quite cold,—otherwise it will fly into pieces. Then it can be laid upon an ordinary glass slide, and examined to see if it is worth preserving, which may be done in one or two ways: first, the glass cover is warmed, and a drop of good spirits of turpentine let fall upon it, covering the diatoms. Just before the spirits evaporate, a small drop of thin Canada balsam is added, and a slide taken, warmed, and a drop of balsam placed upon the centre part of it. Then the cover is brought down upon the slide, the two balsam-covered sides together, in such a way, by tilting the cover slightly, that no air is allowed to come between them, and the cover permitted to fall gradually into place, driving a wave of balsam before it. In this way we have the filamentous diatoms arranged as they grow, but with endochrome removed, which would obscure the markings, and in balsam, which renders them transparent. Some forms, as some of the *Fragillariæ*, become too transparent if put up in this way, and therefore another method of mounting must be adopted with them. They are burned upon the cover, as just described, but mounted dry in air; that is to say, a cell of gold size is made, the glass cover slightly warmed, and then placed upon the cell, with the side upon which the diatoms are fixed, downwards. The warmth slightly softens the gold size, and the cover becomes fixed.

Other forms besides the filamentous species may be mounted in fluid, or burned upon the cover and subsequently put up in balsam, or dry. But the commonest way of treating such forms is to clean them by means of chemicals, as already described, and then previous to mounting them divide the clean gathering, consisting of a white sediment of large and small diatoms along with fine sand, all mixed up together, into densities. Of course, if some of this sediment were to be mounted in this condition, extremely unsightly slides would be procured; so it is best to separate the finer from the coarser diatoms, and these in turn from the sand. This is accomplished by what is known as elutriation, or, separating into densities after the manner already described. Then slides may be mounted from each of the densities in the following manner: A slide is thoroughly cleaned, and a good sized drop of water placed upon the centre portion. A little of the diatom sediment is then taken up in a dip-tube, and the point of the tube brought just into contact with the drop. As soon as a few diatoms have run out of the dip-tube, it is removed. Then a small splinter of wood or stiff bristle is used to disseminate the diatoms through the drop of water in such a way that they will be pretty evenly distributed and not overlie each other. The water is then driven off by heat, a drop of thin Canada balsam placed upon the dry diatoms, and a cover placed on them in the usual manner. In many cases, especially when dealing with the smaller forms, it will be found desirable to mount them upon the cover in this same way, instead of upon the slide, as they will then be brought as near as possible to the objective of the microscope. Single or remarkable specimens of diatoms may be picked out and mounted by themselves; but the manner of accomplishing this would occupy more space than it has been thought desirable to devote to this portion of our subject, and the reader is referred to the books on mounting microscopic objects for the particulars of the process.

The main principles of preparing and mounting diatomaceæ for preservation and study have been given, and the intending student will be able to devise modifications and improvements for himself, so that he will be able to put up specimens in as finished a manner as any to be procured from the dealers.

THE PREPARATION

OF

DIATOMACEÆ.

BY

PROF. CHRISTOPHER JOHNSTON, M. D.

THE PREPARATION OF DIATOMACEÆ.

IN all the range of microscopic research there is confessedly nothing which offers more seductive attraction than that department of botany which comprises the *Diatomaceæ*. Apart from the exclusiveness with which the microscopist makes his observations and pushes his inquiries, there are charms which attach to the life, the modes, and the extent of the reproduction, and to the vast results which follow the multiplication of these organisms. There is also a pleasing bewilderment in their large variety of form and dimension, from the grosser discoids to the almost infinitely little living chambers; and a perpetual delight afforded by their architectural beauty, and by the marvelous and matchless delicacy of the designs sculptured upon the siliceous skeletons of their frustules. The man of science pauses in his work to pore over the tracery of detail, and the philosophic student exhausts resource to effect combinations in objectives and in oculars which shall serve to bring the "markings" into view, and to perpetuate the picture by photography.

However great the interest which life, habits and reproduction inspire, the structure and configuration of the siliceous part especially command attention; for this flinty framework, resisting time and decay, alone endures, and is a recognizable integral in vast strata of the earth's surface, while the softer organic portion, leaving the character of the Diatom to the skeleton, is caught up and utilized in obedience to the law which compels organic matter to incessant action, whether it mount successively higher or fall within the scope of the humblest organism. This animated matter loses its identity, and its relations to particular forms, but the silicified cachet of *Triceratium* and of *Coscinodiscus* is as palpable in the pillared rock or the California stratum as in the recent condition, or in the softer "deposits" of Nottingham, Md., and of Moron, Spain, or in the Guano accumulations of the Chincha and other islands.

"Preparation of the Diatomaceæ," ought strictly to signify the preservation of individuals or groups of these organisms in a permanent way, and their arrangement in a condition suitable for study and future reference. We would begin with the deep sea soundings and end with animated pool-water. It is not our purpose, however, to discuss at present the various devices adopted to accomplish a task so extended; but we desire to point out those methods of isolating the siliceous parts of Diatoms to which experience has given reputation.

It may be worth while to premise by stating—what is, of course, familiar to the student—that the coveted forms are to be met with in a great variety of conditions, either swarming fresh and full of life in pools, ponds, or estuaries, clothed in fibres of green, brown, or yellow, or clustering together in springs, pullulating in lakes and rivers, or tossed by the waves of the great ocean itself. In some of these situations the Diatomaceæ become and are the pabulum of myriads of beings, in whose bodies, as the *Acalephs*, the *Salpidæ*, the *Molluscs*, and the *Holothuridæ*, their siliceous remains are constantly found by the microscopists, who use these and other creatures for their dredgers. They live with and upon other *Algæ*, and are met with in the green ooze of *Confervoids*, and even among the *Muscidæ*.

As ancient or recently fossilized, however, forming strata of considerable thickness, of widely different consistency and density, and not unfrequently of wide-spread geographical distribution, the Diatomaceæ astonish even the workers in science by the extent of their proliferation, and by the uneventful quiet of their living and dying, apparently undisturbed for whole ages in the conditions of their existence. Examples of these tedious and slowly cumulative formations may be instanced in Cassel, in California, in Jutland, and in Maryland and Virginia, the latter furnishing so many varieties of configuration and such rare beauty in design and ornamentation. One of these Diatoms, the *Heliopelta*, so much admired, has been selected by a distinguished author to grace the front page of his admirable work on the microscope.

It must be apparent that no one procrustean method of securing the prize can be made applicable in the business of "preparation." The extreme delicacy of *Amphipleura* forbids the rough boiling which *Coscinodiscus* invites; the free recent forms of any kind in "pure gatherings" obviously require nothing more than the destruction of the organic part, else the fairy-like embossing, as shown in lines or dots, is blurred, or disappears; while the so-called Diatomaceous "earths" or clays often tenaceously resist the deliverance of the imbedded gems, made adherent by a filmy, glassy cement, the product of time, an alkali, and a portion of the seeming lithophytes of other epochs. A lacustrine deposit may be washed out clean with water, but rock must be softened and sulphate of lime removed by boiling chlorhydric acid.

In general, the business of preparation involves two distinct processes: *first*, the liberation of the Diatoms (as we shall henceforth, for convenience, call the siliceous skeletons of the *Diatomaceæ*) from all extraneous matters, with the exception of amorphous silex or some silicates; and, *secondly*, the complete isolation of the Diatoms themselves. The former is, at times, toilsome and disagreeable, by reason of acid fumes which arise in its course; the latter is tedious, and like the other, time-consuming. But both call for a clear knowledge of method and precise executive manipulation, and both demand of the operator an intelligent adaptation of means to an end, and the *patience* with which the attainment of the end is made possible.

The simplest methods of cleaning are not always the most easy,—for example, the rescuing of Diatoms from among the *Polycystinæ* of Barbadoes,—nor the most complicated always the most difficult, as, for instance, the treatment required by a sulphate of lime guano known here as the "Algoa Bay." Let us, however, attempt to make the several methods distinctly comprehensible, although in so doing we run the risk of emulating the tediousness of "neighbor Verges."

Apparatus and Chemical Material. Guided by our experience, the following-named articles are recommended as necessary for the work of cleaning and isolating Diatoms, which should be done in a chamber high above the ground, if possible, and not heated in winter by a flue. Hot-water pipes are far better, as affording immunity against dust. We enumerate:

German beaker glasses of different sizes, with a number of small china plates to serve as covers; several large watch-glasses, or shallow glass capsules of like shape; solid glass rods for stirring; small glass tubes or pipettes; a sand bath and an apparatus for heating; nitric acid, chlorhydric acid, sulphuric acid,—all the best "commercial," except the last, which should be "C. P.;" carbonate of soda and carbonate of potassa, both C. P.; Atkinson's alcohol; freshly distilled water, and a copious supply of filtered soft water.

For displaying a cleaned sediment with a view of securing individual specimens, a number of glass slides one and a half inches by four inches should be provided; and for the preservation of finished work, or clean Diatoms, a score of small bottles, with corks already fitted.

Finally, a large camel's-hair pencil; a few slender cane (reed) strips, to serve eventually, when pointed very finely, to pick out single valves; a supply of litmus paper; a glass funnel, and Saxony filter paper, complete the category.

Method to be Employed. As pure Diatoms, guano findings, and diatomaceous earths or clays each require separate modes of treatment, let us first handle a guano specimen, because some of the steps to be trodden may be called fundamental, or we may say they are of very general application: still, they must in certain instances be preluded by others, may not be wholly needed in particular cases, and are of necessity to be followed by supplementary processes demanded by the peculiar nature of the products obtained.

A guano, such as the Chincha or that of Ichaboë, ought to be coarsely sifted to free it from pebbles, feathers, and masses of crystallized substances. The better part is still, however, very heterogeneous, consisting of Diatoms in a very small percentage, and of much extraneous matter, earthy, salty, and excrementitial. Boiling water dissolves a great part of all these, and should be repeatedly applied to the deposit, and as often suffered to stand after stirring, so as to leave behind the insoluble constituents, among which, of course, are the objects of our search.

The sediment will be materially lessened in bulk by a good boiling in a solution of carbonate of soda, one ounce to the pint, which dissolves much organic as well as some inorganic matter, and, besides, sets free adherent Diatoms without injuring their structure. Carbonate of potash, however, is not so free from objection.

The residuum, being drained upon a filter, ought now to be boiled in an equal-part dilution of nitric acid for about ten minutes in a beaker glass, the quantity of the fluid being a couple of inches in height above a half-inch of the matter upon which it is destined to act. Lime not in the form of sulphate, and some other elements, are dissolved out as nitrates, and must be poured off in the solution when cold after standing. Hot water should now be added freely to the sediment and poured off after its subsidence, until all acid shall have been removed, whereupon the residuum is to be once more drained upon a filter.

The matter remaining is now ready for pure nitric acid, in which it is to be boiled for five or even ten minutes; after which treatment, and before cooling, the whole must be deluged with hot water. After standing, the supernatant liquor is to be poured off, and the refractory deposit washed clean with cold filtered water, and drained as before stated.

The sediment now, much reduced in quantity, is prepared for chlorhydric acid, in which it is to be boiled for the removal of sulphate of lime if in moderate quantity, perhaps of a small percentage of other matter, and of such metallic stains as have resisted the action of the aquafortis. Besides, the chlorine has bleached such vegetable organic *débris* as have escaped destruction, so that the sediment, now composed of Diatoms, fine sand and siliceous dust, and extraneous vegetable remains, appears of a pale gray color.

When thoroughly washed in filtered or distilled water, and then rendered as free from moisture as possible, either by means of a filter or by gravitation, the deposit must be subjected to the action of sulphuric acid, C. P., which, heated to the boiling point, carbonizes the vegetable matters, which, in a charred state, blacken the fluid. The removal of this carbon is to be accomplished in the form of carbonic acid by the addition to the still boiling acid of oxygen, which, at the very high boiling point of sulphuric acid combines with it, and the gas escapes in ebullition. Nitric acid (NO^5) may be slowly poured in until the black or dusky color gives place to the orange hue of nitrous acid (NO^3), being what is left of the nitric acid that has parted with two elements of oxygen; or else chlorate of potassa in fine powder, after the manner of Bailey, may be very gradually, and in small doses, dropped into the seething liquid. Upon each contact of the powder a vivid explosion takes place as the carbonaceous particles ignite and consume. Chlorine is evolved, to the great annoyance of the operator, and sulphate of potassa is added measurably to the sand and Diatoms beneath, But soon all is of pearly whiteness, and the process is at an end.

The task of cleaning is near its accomplishment; for all that remains to be done is the abstraction of the acid and the washing out of the sulphate of potash.

Let the tyro be careful, and manipulate with deliberation; for the rapid admixture of sulphuric acid and water occasions a sudden and considerable rise of temperature. Instead of pouring or drawing off the hot or cooled fluid, we would recommend that a large beaker glass two-thirds full of hot filtered or distilled water be made ready, and that into the water, by very tardy pouring, or even dropping, the acid, and all it contains, be thrown. When cold, or nearly so, the supernatant fluid must be flowed away, which process is facilitated by holding a glass rod against the beaker's edge to guide the stream, and after repeated washings with distilled water in a fresh beaker (for the sulphuric acid clings to the pot-beaker), the Diatoms, the sand and the amorphous silica alone survive. All is now perfectly clean; but the constituents of the white powder must await separation, and this they can do only in *dilute alcohol*, because the particles mat or adhere irretrievably in water, in which, also, confervoids speedily arise. We *label* the vessel, and we choose our own time for isolating the precious forms, observing, however, that the whole sediment has shrunk within very small dimensions, as we set aside the result of so much labor.

Finally, in reviewing the work done, let us have in mind the intention of each of the acids employed, and remember that NO^5 boils at a moderately high temperature, which, as in other fluids, is decreased by the presence of pebbles, bits of glass, or coarse sand; that HCl passes into ebullition at a comparatively low indication of the thermometer; and, lastly, that the boiling point of SO^3 is very high indeed,—so elevated, in fact, as to jeopardize all inferior glassware.

The foregoing process is open to the charge of being time-consuming,—as are all other methods,—but we have invariably found the results to be excellent. The same success is claimed for a different procedure, practiced and recommended by F. G. Stokes,* and which may be here briefly set forth.

Provide a beaker glass of six or eight ounces' capacity, in which place about two teaspoonfuls of guano, and then fill to near the top with a saturated solution of carbonate of soda. Boil for half an hour, wash well with water, and, after standing, pour off the supernatant fluid very close.

Add now of chlorhydric acid two ounces, boil also for a half-hour, wash well, and pour off very close once more.

Treat the sediment with one ounce of strong sulphuric acid. Let it act for ten minutes, and then add bicarbonate of soda cautiously, either in solution or suspension. Shake well during effervescence, wash well, and, with great caution, add two ounces of nitric acid. After effervescence, drop in two pinches of chlorate of potash, boil a half-hour, or until the deposit becomes white, and, finally, wash the sediment thoroughly.

From what precedes, it must appear that the aim of the operator is the removal of all inorganic substances, either originally soluble or artificially made so, prominent among which is *lime*, and of all organic matters reduced by a destructive process to a soluble or gaseous form. And it is also evident that when certain of these stranger elements are known to be absent, such parts of the processes as are applicable to them ought to be omitted, so that fewer steps are necessary to attain

*F. G. Stokes, On Cleaning Diatoms, "Quarterly Journal Microscopical Science," Vol. XV, p. 222.

the end. Silex, or sand, of course, is not to be regarded in this connection, as it is as insoluble as the Diatoms themselves.

Suppose we take a clay or earth, that of *Nottingham*, for example. Cretaceous matter forms but a small part of its substance, which consists, in fact, of Diatomaceous skeletons, more or less adherent through the agency of a mortar, probably a silicate of lime, and of fine siliceous particles, or even sand, less closely connected. To disintegrate a mass, let it first be slaked, as it were, by pouring over it a strong solution of carbonate of soda, and when, after a time, the whole falls to pieces in laminæ and in dust, let it be boiled for fifteen or twenty minutes in a quantity of the same solution, and the result will be the reduction of the cement, the formation of silicate of soda and carbonate of lime, and the almost perfect cleaning of the Diatoms. The former is removable by hot water and frequent washings; the latter, by boiling in nitric acid; while chlorhydric acid dissolves out any sulphate of lime, and, besides, bleaches by the abstraction of unattacked metallic stains. The fine siliceous dust, the torment of diatomists, can only be gotten rid of by elutriation, as will presently be shown, and refractory particles or lumps must be left behind in the washings.

If the reader have followed our *procès raisonné*, he will hardly be at a loss to answer the query, "What are we to do with such clay or rock as the *Monterey?*" some specimens of which we have found to be extremely hard or tenacious. In this instance, again, the difficulty presented is the disintegration of the rock without doing injury to the Diatoms. We may make the mass very hot and then drop it into cold water, by which many Diatoms will be sacrificed; or else we may slightly warm the specimen, drop it into a strong solution of carbonate of potash, and boil for a time, to be ascertained by the breaking down of the original lump. In the same way, carbonate of soda may be employed, with more safety to the forms in request, but with less general success; while the potash, which is more energetic in decomposing the cement, is very destructive, if not carefully watched, of the very objects we seek.

Once reduced to the state of powder, the rules just enunciated are to be followed.

Before leaving the difficult or troublesome, we feel obliged to notice such guanos as contain sulphate of lime in any quantity, but especially in large proportion. *Algoa Bay Guano* (South Africa), for instance, as furnished us by a reliable person, was found to consist almost wholly of this refractory substance, which required an especial treatment. Being soluble in boiling and hot dilute chlorhydric acid, it was found necessary to boil the Algoa guano in that dilution, and to pour the whole on a filter, whereby the sulphate of lime in solution ran off and deposited on cooling, while the guano residuum was caught by the filter, to be subjected again and again to the same process as long as it contained the salt of lime. The small portion of decalcified guano was next exposed to the action of NO^5 and HCl, in the usual way, with the result of securing some beautiful *Actinoptychi* and *Aulacodiscus Petersii*.

Pure gatherings, unmixed with sand, mud, or other refractory extraneous substances, are not made to pass through the ordeal of an alkali, and so many acids, as in the case of a guano, but may be cleaned by the use of one of these agents with the aid of no long-continued heat. Chlorophyll will yield to carbonate of soda, and all possible lime here, to nitric acid; but when we have to deal with very delicate *Amphipleuras* or *Grammatophoras*, marked with almost ghostly lines, we should handle our reagents with gloves, and not boil out those exquisite markings which almost rival the art-ruled bands of Nobert. Maceration for a time may be sufficient; or, if the quantity be small, all phytic substance may be burned away by the heat of a spirit-lamp flame applied beneath a film of mica, on which the "once animated dust" reposes, and on which it may, without change, be mounted in balsam.

It is needless to remind the young operator of the necessity for delicacy in all manipulative procedures, and not least in the washings of pure gatherings, or of those containing the filmy forms. But care is especially to be exercised in the recovery of those most fragile Diatoms which are met with in *Barbadoes earth*. Water alone, poured *gently* on this polycystinous deposit, will suffice to float away and waft them to slides ready for their reception. And this may be done in a beaker, so that the supernatant water, rendered milky by diatoms and siliceous dust, yields by elutriation the fairest of results.

Isolation of Diatoms.—We have already pointed out the advantage, nay, even the necessity, of preserving the fruits of all cleanings in dilute alcohol, in which they may rest in safety awaiting the separation of the morphous from the amorphous. And we may here add the advice to recommit the Diatoms to alcohol finally before mounting, or incidentally, if interruption temporarily arrest the perfect course of the isolation. The methods of Mr. F. Okeden* by decantation, and by whirling in an evaporating capsule or large watch-glass, as suggested by Mr. J. A. Tulk,† we have found to answer every requirement if they be as dexterously managed as they are ingeniously devised. But we would call attention to a point, not hitherto noticed, incidental to the decantation process, and which has reference chiefly to the discoid forms. It is this: When the Diatoms were nearly or quite free from foreign matters, and beaker glasses were being used in the preparation, we observed that entire disks adhered to the flat bottoms of the vessels. We utilized this knowledge by emptying a beaker, by washing it out quite freely with distilled water, and, finally, by detaching and collecting the absolutely perfect Diatoms by means of a soft camel's-hair pencil, well cleaned. In this way we have had excellent success with many gatherings, but with none better than the *Nottingham earth*, as was shown by some of our slides exhibited in Chicago in March, 1871, at the reunion of the State Microscopical Society of Illinois.

We have now, by whatever process employed, attained the cleaning and separation of the Diatoms, and have consigned them to the temporary guardianship of dilute alcohol; but there still remains for us the task of a final preparation of them for mounting. By the plan adopted and suggested by Mr. Okeden, they have been "sorted" as to size; yet one washing more is necessary before we can transfer them to the expectant slides. If the Diatoms were to be dipped out by a tube directly, and dropped either upon a cover or a slide, the rapid alcoholic evaporation would keep the whole field in agitation, and the objects would eventually group together in drying, and

*Method of Washing Diatomaceous Earths and Clays; "Quarterly Journal of Microscopical Science," Vol. III, p. 158.

†On the Cleaning and Preparing of Diatoms, Vol. XI, No. 3, III.

materially mar the beauty of the preparation. This defect may be easily remedied by quickly washing out the alcohol; after which the even display of the diatomaceous forms is readily accomplished, especially if we give the end of the slide a fillip with the finger previous to putting it aside to dry spontaneously. At this point the microscopist has the election either of mounting in the *dry way*, or of embalming his tiny treasures in Canada balsam. He may add the balsam drop *au naturel* and gently heat over a spirit-flame before applying the cover, or he may omit the evaporation and mount "soft," placing a small screw, for a weight, upon the cover, or he may use balsam thinned by chloroform, or dissolved in absolute alcohol, and filtered, as recommended by Dr. Schaeffer, of the Army Medical Museum, and then apply the continuous pressure of a small weight. By the first method the slide will be immediately ready for use when labeled; by the others, a certain number of hours or days must elapse before the margins of the covers will have become securely attached. But the work is done, and the student may now gloat over the things of beauty which he views by the light of science. It is true that he may regret a few forms which have been floated off under the descending cover; but careful manipulation alone will in future prevent this mishap. But how can he imitate the exquisite groupings of the *Diatomaceen-Typen-Platte* of Möller? He may not equal, but he may, with much practice, approximate to the excellence of that wonderfully skilful preparer by arranging his Diatoms dry upon a slide or cover previously coated with a thin film of gelatine, and then fixing them by exposure for a moment to the vapor of distilled water. In mounting with balsam the Diatoms cannot change position, but the gelatine disappears, and is seen no more forever.

Before closing this paper, perhaps already too long, we deem it germane to the subject to refer to the plan which we have adopted with success in the selection or picking out of particular Diatoms. It may not be altogether original, yet it is practical, and may aid the inexperienced.

Nothing is easier than to seize particular diatoms and transfer them to a bottle for future use, or to a slide, provided the field from which we select be rich and clean. Difficulty, however, occurs when forms in any gathering are few and far between. Let such prepared material be spread upon a large slide, covering a space of one inch by two, and let it be filliped as it is set away to dry spontaneously. With a two-third inch objective, search the white field for any Diatoms whatever, and, upon finding, encircle each one with a line, made with a point of a match sharpened and moistened, adding near the circle a dot, or cross, or other sign, always appropriated to the same Diatom, and of which a tallying record is kept on paper. At leisure one may, without trouble, single out any desired object, pick it off with a fine dampened point of cane (reed), not including the siliceous cuticle, and deposit it, free from injury, in a small drop of distilled water placed in the centre of the slide.

And here we leave our subject, with the remarks that none of the methods proposed can lead to success unless aided by patience, pains-taking, the adaptation of means to an end, and by practiced manipulative skill; and that what appears to be present perfection is only to be regarded as one of the widening circles which tend towards, but never reach, ultimathulan truth.

ON

THE PREPARATION

OF

DIATOMACEÆ.

BY

PROF. HAMILTON L. SMITH, LL.D.

ON THE PREPARATION OF DIATOMACEÆ.

UNDER the above heading, Christopher Johnston, M. D., has published a very excellent article,* to which the following paper is intended as a supplement, and I know of no better guide for the student. What I have to say relates to the rapid preparation, from crude material, where this has been at all carefully gathered, and to a mode of mounting, *invariably on the cover of the slide*, not mentioned by Dr. Johnston, but which has some great advantages. The gatherings should not be dried, but kept moist, in phials, with a little creosote to prevent mould. I very much prefer to examine whole frustules, with both valves adherent, or if filamentous, still cohering. And I have many bottles of preparations for mounting which are nearly as clean as though they had been treated with acids. And many of the most interesting preparations which I have were never boiled in acids. Of course, very much depends upon the skill and carefulness of the gatherer, and a little patience and judgment will enable any one to obtain the crude material tolerably pure. Only a few days ago I made a gathering of *Nitzschia*, in which I have the frustules almost as free from foreign matter as though they had passed through the most elaborate acid and chlorate of potassa treatment.

Supposing, then, that one has before him a phial which will hold a considerable quantity of water compared with the sediment in it, the latter composed more or less of diatoms. We

*Reprinted on preceding pages.

proceed thus, and if it has stood for some days perfectly undisturbed so much the better. The bottle is twirled rapidly, and the lighter material rising up in the axis will soon diffuse itself throughout the water. Allowing it to settle for two or three seconds, until to the eye the grosser portions have just been deposited, all that remains floating is now poured off into another phial, and it is from this stock that we are to separate the diatoms and sand from the clay and organic matter. The material poured into this second bottle is allowed to settle until the water simply appears milky or cloudy; the time will vary according to the minuteness of the diatoms, and can only be judged of from experience, say one minute, when all that remains floating must be poured off, and thrown away, unless there are very minute forms which it may be desirable to separate. The phial is again to be filled with rain, or distilled water (hard or lime water should be strictly eschewed), and again shaken up. As soon as the heaviest deposit touches bottom, the rest should be poured off into a third phial, leaving say about one-fourth the amount behind in the second phial. This third phial will now consist mainly of sand and diatoms, with lighter organic matter and pure clay; the last two can be removed by elutriation; for this purpose, fill the phial No. 3 with water, and after well shaking allow it to settle two to five minutes, pour off and throw away the slightly milky water, and repeat the operation, allowing it to settle a somewhat longer time; the operation may be repeated a third time, when particles, suspended after an interval of eight or ten minutes, may be poured off. Often, after the first settling of bottle No. 2, the diatoms will rise more pure in the mass by twirling the bottle than by shaking it up. A little practice and care will enable any one to separate certain diatoms according to size. I had a gathering of *Pleurosigma Spencerii* from Scioto River, O., sent to me, but although it had been chlorated, still, when a mounting was made, not more than one or two frustules would be in the field of view, the great mass being either smaller forms, or fine fragments of silex; by careful watching and testing the time when the different sizes would remain suspended, I have made from this a preparation, which will show hundreds where before were scarcely any, and which would never be recognized as the same gathering. Supposing now a trial shows us the diatoms tolerably abundant, the trial being made by heating in the manner presently to be described; the phial is filled with alcohol and water, half and half. Some samples of alcohol leave behind a scum after evaporation, especially noticeable after burning in the mode presently to be described, and water which will leave crystals, or any scum, must be avoided; the beauty of the preparation will largely depend upon being particular in this matter.

For mounting diatoms I invariably place a drop of the fluid containing them upon the cover, *never on the slide*. The alcohol and water will spread out on the slide, but will remain heaped up on the round cover, like a plane convex lens. I prepare a little stand, represented in the accompanying wood cut, of quite fine wire (so as not to conduct off too much heat) bent at right angles and inserted into a base; the free end is bent into a ring, and upon this ring is placed a square plate of very thin iron, (such as is used for the so-called "tintypes" in photography, with the Japan burned off,) held in place by bending the corners of the square over the ring, loosely, to allow expansion, without bending when heated; upon this plate the cleaned cover is placed, and then, by means of a pipette, a drop of the alcoholic liquid with the diatoms is placed upon it, and the spirit lamp applied below. The alcohol takes fire and is allowed to burn out; the flame of the lamp is then placed beneath, and the rest gently boiled, the remaining alcohol escaping during this ebullition causes the diatoms, by this very act, to distribute themselves very evenly over the cover, and all matting is effectually prevented. It is better after one perceives that this even distribution has taken place, not to push the heat so as to make large bubbles again, but to slowly evaporate until dry, after which the full power of the flame must be applied until the iron plate and the glass cover is red hot; at first the mass of diatoms, etc., will become black, but as the organic contents and *debris* burn away there will finally remain only the silex nearly white. I invariably burn in this manner on the cover even the specimens which have

been prepared with acids, for the diatoms thus treated when mounted appear much sharper and cleaner. The amount of heat, if the diatoms are rigidly siliceous, as most of them are, may be the full power of an ordinary alcohol flame continued for some time, but if they are imperfectly siliceous, care must be exercised in the burning.

I invariably use old balsam for mounting, just as bought from the shops, especially if I wish to have a specimen which will bear immediate handling, or be ready to be sent off soon as mounted. Allowing then the cover to cool, while the slide is being cleaned to receive it, I place a drop of the balsam, which must not be fluid, only viscous, on the middle of the slide, and now *with this* pick up the cover from the little stand where it has been heated. The diatoms will be so fastened by the heating, that but few will flow out from under the cover, if any, in the subsequent treatment. I now hold the slide over the flame of the lamp (which should be much smaller than when used for the burning) until not only all under the cover is a mass of small bubbles, but until very large bubbles, balsam steam, appear; the flame is removed soon as the bubbles are observed all running to one edge. I press down the cover at this place by a mounted pin, and start them in the opposite direction. This may seem unnecessary, but long experience shows that this is the better way to get rid of them; during this the slide is held somewhat obliquely, the cover is kept from slipping by the pin, and if all the bubbles do not disappear, then with a very small flame heat is applied just beneath the obstinate ones, the slide being held slanting, and that part upwards where the bubbles are nearest the edge of the cover. The description is longer than the actual process, and the slide when cool is ready for immediate use. Perhaps I am wedded to old ways, but after trial of fluid balsams, without heat, I have always come back to the old way; still, for selected diatoms, some of these preparations of balsam are good. If the diatoms are to be mounted dry, always the best way, if for real study, I make a ring of the zinc white in balsam, (sold by the opticians,) and which in a moment or two is sufficiently hard to receive the cover, *and never runs in;* after standing an hour or two I give a finishing ring of same, or the usual black varnish on the outside.

I think any one who will adopt the mode of mounting on the cover, and subsequent heating, as above described, whatever may be the rest of the procedure, will never consent to give up this part, since it effects so even a distribution, and such destruction of residual organic matter, and gives such increased brilliancy to the preparations; sometimes, if the acid has not been thoroughly washed out of acid treated specimens, snappy explosions will occur when the alcoholic mixture is heated; of course, the remedy is to pour off, and replace with pure water and alcohol.

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