Danmarks Geologiske Undersøgelse.
IV. Række. Bd. 3. Nr. 7.

Serial Sections of Small Fossils.

A New Method.

By

Jørgen Birket-Smith.

Med dansk resumé.
With 1 Plate.

I Kommission hos
C. A. Reitzels Forlag
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KØBENHAVN

Contents.

Preface 7
Historical 9
Older Techniques 12
Author's Serial Section Technique 15
Appendix (Materials) 24
List of technical words 26
Dansk resumé 28
Bibliography 29
Index 31
Outline of the Two Serial-Section Techniques... Plate

Abstract.

An historical review of the practical study of foraminifera is followed by a selection of detailed accounts of methods for the study of the internal structure of foraminifera. The author's technique for serially sectioning small fossils is presented in great detail. After embedding in celloidin the fossil material is dissolved, and replaced by paraffin wax which may, after microtoming, be removed, and the celloidin stained and embedded in dammar-xylene.

This technique enables the finer detail of small fossils to be studied in transparency. Photographs of completed preparations, and a comprehensive bibliography are included.

Preface.

The following pages are in part an attempt to review the old methods for the study of small fossils, particularly the internal structure of foraminifera, and in part an account of a new method by which I have tried to solve the problem of serially sectioning small fossils.

With regard to the first part I must say that there can well be methods which have escaped my attention, but I hope that I have included the most important. In each case I have tried to trace back to the original publication of the method, and to give the "recipe" correctly even if sometimes in abbreviated form. All the original publications and a few comprehensive works on technique are included in the bibliography.

I got the idea for the new method during my work on the Geological Survey of Denmark, and the actual development of the method took place in the Institut for Almindelig Zoologi (Institute of General Zoology) in the University of Copenhagen. Here I must thank Prof., dr. phil. H. V. Brøndsted for permission to use the Institute's laboratories, and also dr. phil. N. Thydsen Meinertz, dr. phil. P. J. Holst-Christensen and dr. phil. C. Overgaard Nielsen for their kind assistance. I furthermore thank Mr. P. Baadsgaard, B. Sc., for the translation.

Finally, I must express my thanks to the Director of the Geological Survey of Denmark, dr. phil. H. Ødum, who has made it possible for me to complete the experiments and publish the result.

Copenhagen, September 1949.

Jørgen Birket-Smith.

Historical.

The first time foraminifera are mentioned in the literature is, as far as I have been able to discover, in 1731 when BECCARI found these small shells which he considered to be Horns of Ammon (*cornu hammonis*) during a microscopic investigation of the sand at Bologna, and therefore decided that this sand was of marine origin. He describes briefly the exterior of the shells and also mentions the internal division into chambers, concerning which he writes (p. 67): ". . . . . hæque, fricatu sæpius levi quodam corpore non sine aqua & minutissima arena planiori testæ parte, donec id, quod in nodis eminebat, disrumperetur, facile & sine dubio apparuerunt." It seems as though BECCARI ought to be honoured not only for having discovered foraminifera, but also for having introduced grinding as an assistance in the study of their interiors. A technique which, strangely enough, but perhaps partly because of the slight knowledge of BECCARI'S work, was immediately forgotten.

G. P. S. BIANCHI is the first who has depicted foraminifera. In a work published in 1739 under the pseudonym "JANUS PLANCUS" ten foraminiferan tests with the name *Cornu Hammonis*, together with several other small shells, are illustrated in copper plates. Although it is impossible to determine any of the species, the pictures, which are fairly good, indicate a thorough investigation. The internal structure is nowhere shown, but the division into chambers is described in the text.

LINNÉ (LINNEUS) was also aware of the internal structure of foraminifera, as he includes several in the genus *Nautilus* because of their internal division into chambers (1758). This knowlege is perhaps derived from accidentally fractured tests; in any case he nowhere, not even in the little work of 1772 on the arrangement of the naturalist's cabinet, writes that he has artificially opened any to examine the interior.

F. H. W. MARTINI (1769) is aware of this common feature in the internal structure of foraminifera, but nonetheless he separates the

uncoiled forms such as *Nodosaria*, etc., from the rest, and, since he only utilizes the external features, includes them with *Pectinaria*, *Dentalia*, etc., in a group, "Sea Pipes". He remarks, however, (p.5): "In Ansehung ihrer innern Bauart haben die kegelförmigen Meerröhren mit kammern eine grosse Aehnlichkeit mit den Schiffkutteln und Ammonshörnern, so sehr sie auch in der äussern Form von diesen abweichen."

MARTINI shows in an original engraving (p. 1) several uncoiled foraminifera, *Nodosaria*, *Vagulina*, etc., of which some are opened so that their interior is visible. These are undoubtedly opened by cutting ("durchgeschnitten"), as he remarks that this is very difficult to accomplish without crushing them—in which one must agree with him. A single one of the coiled forms (Pl. XX) is certainly cut open, and an other has a part of the wall removed so that the interior is visible, but from a remark on p. 262 one must believe that such specimens are great rarities. He is aware of the openings between the chambers ("Nervenröhr"), but has not detected pores etc. in the test.

A. J. G. C. BATSCH re-introduced the grinding of foraminifera, as in 1791 he mentions with the expression "ausgeschliffen" some shells that are pictured opened. Nowhere on these otherwise excellent illustrations are shown actual pores in the test material, but one has the impression that BATSCH was aware of a certain porosity in some parts of the test.

I have Unfortunately neither had opportunity to see any of the models of foraminifera which A. D. D'ORBIGNY made in 1823 (no doubt the first attempt to reconstruct these attractive animals), nor have I seen the original catalogue of the collection. From a reproduction[1]) of the text of this catalogue it is not apparent how the models are made, and it seems as though it has only been attempted to portray the exterior of the tests. D'ORBIGNY'S own account of the models in 1826 contains an indication that they are free-hand models. The growth of the chambers is described, but neither pores, nor canal systems.

First in 1848 did the investigation of the finer structure of foraminiferan tests begin to advance rapidly, as W. C. WILLIAMSON, the 20th. December 1848 (printed 1849), published some investigations

[1]) PARKER, JONES and BRADY: Ann. & Mag. of Nat. Hist., Vol. XVI, 3rd. Ser., 1865.

of "*Polystomella crispa*" where for the first time information was given on thin sections of foraminiferan tests.

Unfortunately WILLIAMSON does not describe how he prepared these sections. He worked with fresh material, and it is most likely that he made thin sections by grinding, even though no such sections are illustrated. This is the more likely as W. B. CARPENTER in 1849 writes: "Applying to these fossils the same method of investigation as that which had been so successfully employed in the case of teeth, bones and shells — —", and pictures a number of thin sections. This method had already long been used by histologists (LOUIS MANDL, RICHARD OWEN and others, 1839 ff.).

In 1859 CHRISTOPHER JOHNSTON published accurate instructions for the making of thin sections, "so that students should not have to buy expensive finished preparations of fortuitous objects."

In the meantime, CARTER, an English doctor in Bombay (1852)[1]) discovered the technique of filling foraminiferan tests with carmine by capillarity, followed by evaporation to draw the stain into all the pores, thereby making them distinct. A technique which has since been used and varied time and time again (BROTZEN 1936).

In 1880 MÖBIUS published a combined grinding and staining technique in which he coloured the thin sections when finished, obtaining eminent results with regard to pores and canals. BEISSLER reached a rather more comprehensible result when he published, in 1891, a method by which he produced artificial casts of foraminiferan tests which, after the test was etched away, showed all cavities and canals as solid. This was an important advancement of that method of investigation which EHRENBURG had published in 1855, in which one etches away the calcite of natural stone-casts of foraminiferan tests.

A machine, or rather two, for the production of series of thin sections was constructed by C. F. BÖDEKER (Publ. 1921), but as the object has first to be sawn into plates with a 0,5 mm thick aluminium disc armoured with emery or carborundum powder, its use in foraminiferal technique will be strictly limited, and in any case useless for reconstructions. The method can, however, be used with advantage in the investigation of compacted foraminiferal rocks.

An other method of serial sectioning was invented by BÖDEKER (Publ. 1926), but this can only be used for recent foraminifera with well fixed protoplasm in the tests as calcareous matter is comple-

[1]) EHRENBURG 1855.

tely removed, and only the organic components remain. In the same year (1926) J. HOFKER published a communication on how he had investigated pores and canals in thin sections of fossil foraminifera by etching away the calcite after embedding the thin sections in canada balsam. This method, which shows the same as that of MÖBIUS, is much easier to effect, but according to RUTTEN (1936) the fine threads of canada balsam laid down in the finest pores cannot support themselves after solution of the test. He recommends thin sections of tests which are impregnated with iron compounds.

Of the particular technique for arenaceous foraminifera must be mentioned that LECRIST (1934) recommends that, while such a water filled test dries the water surface in the cavities should be observed under the microscope, and an impression of these thereby obtained.

Older Techniques.

CHRISTOPHER JOHNSTON's "simple and certain method of preparing and mounting tissues such as bone, teeth, shells, fossilized wood, &c."

The object is first cut into plates with a thin saw. The plate which it is desired to grind can be held in the fingers. If the object to be ground is very small it is attached with gelatine to a piece of glass which should have a piece of very thin paper glued over it, and it is protected from over-grinding by being encircled by a ring of thin card. Grinding is effected first on the plane wet surface of a coarse Kansas hone, after which the ground surface is washed, and ground wet on a fine Kansas hone, finally to be polished on a soft linen cloth resting on an even base.

Next, a piece of glass is covered with thin paper attached with gelatine, upon this is attached another piece no more than 1/500th. inch (circ. 50 μ) thick which has an aperture cut in the middle through which the already polished surface is cemented—also with gelatine—onto the lower piece of paper, after which the whole is dried under a light pressure. A pair of black pencil lines on the paper under the object to be ground make it easier to see when it becomes sufficiently transparent. The object is ground and polished as before, but in oil.

The section is quickly freed by immersion in warm water, cleaned

thoroughly on both sides with a camel-hair brush and soapy water, rinsed and dried. When the section is quite dry it is placed in chloroform and examined for possibly adhering particles, and is then ready for mounting. Sections which have a tendency to warp while drying may be dried between two glass plates.

When the section is to be mounted a layer of canada balsam is melted on a slide, and as soon as the balsam is cold the section is laid on this and covered with a drop of melted balsam, after which the whole is warmed carefully, and, when all air bubbles have been removed a clean cover glass is laid in place.

This method has been much used, and much varied. For foraminifera it can be recommended that, before grinding, the whole test be embedded in canada balsam, or even better, in a mixture of 16 gm. canada balsam and 50 gm. shellac (ROSENBUSCH). In place of grindstones glass plates with different grades of carborundum powder can be used with advantage if one is not lucky enough to have a rotary grinding-plate, of which there are several patterns with fitted object holders etc.

MÖBIUS's Stained Section. The thin sections are polished on lithographic stone, and after they have been cleaned are laid in alcoholic fuchsin solution for 12-24 hours, after which the fuchsin is precipitated with water. Stain on the surface is removed by a few strokes on the polishing stone, after which the section is air dried and mounted in canada balsam or better, in shellac.

Artificial Stone Casts in Foraminifera and Bryozoa (P. BEISSEL, 1891). The well cleaned calcareous tests are placed in a solution of waterglass which is saturated with silicic acid by the addition of an excess of silica gel. After slow warming, by which means the air is driven out of the tests, the waterglass solution is evaporated to a syrupy consistence, preferably at room temperature, and with occasional stirring to prevent the formation of a skin on the surface. The tests are removed with a brush and placed in ammonium hydroxide with admixed copper sulphate. When they are saturated with this the solution is neutralized with hydrochloric acid without solution of calcite, the fluid is evaporated and the tests are taken up; when they are washed quite clean the process is repeated. The whole process is repeated three times, after which the tests are placed in a watchglass with water, and acid is added drop by drop

until the calcareous matter begins to dissolve. The separation of carbon dioxide must take place very slowly, so that the casts are not damaged. When all calcareous matter appears to have dissolved the casts are placed in strong acid to remove possible calcite remains. They are then washed in water and placed in alcohol which is carefully warmed to drive out air. The stone cast is now completed, but very fragile; it is embedded in canada balsam on a slide.

C. F. Bödeker's Celloidin Section Technique for the demonstration of small amounts of organic matter in tooth enamel, calcareous sponges. etc., can be used for well fixed recent foraminifera since only the protoplasm remains after treatment. The fixed animals are taken through the following fluids:

1. Alcohols of increasing concentration to dehydration (10 mins. each.)
2. Methyl alcohol (1-2 hours)
3. Acid celloidin (20 cc.)

The animals are decalcified in a mixture of 150 cc. of a syrupy solution of paraloidin in chemically pure methyl alcohol, to which a mixture of 10 cc. nitric acid and 40 cc. methyl alcohol is added drop by drop while stirring. The mixture contains about 5 % nitric acid, and the animals must remain, without being shaken, in this for 1-8 days before the calcite has completely dissolved. The extent of solution can be controlled under the microscope, and to minimize evaporation the mixture must be kept in a closed vessel.

4. The celloidin is hardened by taking off the lid of the vessel. The animals are cut out with a small rim of hardened celloidin about them, and are placed in
5. 70 % alcohol for 1-2 hours
6. 40 % alcohol for 1-2 hours
7. Aqueous alum solution for 24 hours, to neutralise the acid
8. Running water 6-8 hours
9. Alcohols of increasing concentrations up to and including 95 %, each 1-2 hours (Abs. alcohol dissolves celloidin)
10. Aniline oil for 6-12 hours to remove the remaining water. The celloidin thereby becomes brown and quite transparent
11. Aniline-chloroform in equal parts, 3-6 hours
12. Chloroform 6-12 hours.

In preparation for sectioning the celloidin blocks are embedded in paraffin wax.

13. Paraffin dissolved in chloroform at about 20° C, 3-6 hours
14. More paraffin is added and the solution remains for 3-6 hours at about 38° C
15. Pure melted paraffin (melting point not more than 52° C) in a thermostat for 2 hours
16. The paraffin with the celloidin block is poured into a mould and cooled quickly in running water
17. The paraffin block is trimmed and microtomed at 3-10 μ
18. The sections are laid on a drop of water on a slide smeared with egg-albumin-glycerine
19. The slide is kept at 38° C for 24 hours so that the sections may stretch and adhere to the glass as the water evaporates
20. The slide is placed for 3 mins. in xylol to dissolve the paraffin
21. Ether-alcohol to remove the celloidin
22. Absolute alcohol to remove the ether
23. Alcohols of decreasing concentrations, each 1. min.
24. Distilled water 1 min.

HOFKER's Canada Balsam impregnation[1]). A thin section, made in the normal manner, is mounted in boiling balsam on a slide. When the balsam is cold and hard the slide is placed in acid where the calcareous matter is dissolved and the canada balsam, which filled even the finest pores, remains.

Author's Serial Section Technique.

After a series of experiments I have decided upon the following method for the production of serial sections of small fossils such as foraminifera (both arenaceous and with calcareous tests), bryozoa, echinoderm spines, etc. It must at once be remarked, however, that the method is rather exacting, both with regard to materials and care. In the following I will try to give so careful an account that anyone, without particular previous knowledge of micro-technique, can use it.

The material must be clean both outside and inside. It can be freed from loose particles by boiling in sodium carbonate or hydrogen peroxide (p. 26). Internal cavities as well as the external

[1]) After "Foraminifera of the Siboga Expedition" as the original account in Natuurhist. maandbl., Maastricht, is not publicly available in this country.

surface must be free from pyrites; whether they are is best seen by examination in transmitted light while the fossil lies in clean xylene (with regard to possible further treatment the xylene must be absolutely clean).

I have fortunately been able to remove quite small amounts of pyrites (see fig. 3) by allowing the test to remain in a bath of 10 % hydrogen peroxide, 10 % ammonia and 10 % potassium carbonate, which is renewed each day. The bath does not damage the carbonates, but solution of the pyrites takes place very slowly, and the process is arrested after about 14 days. I have been unable to find any reasonable explanation of why the process stops, or even of why it starts.

The completely clean fossils are thoroughly dried in an oven at 110° C for 24 hours; they are then placed in absolute alcohol for 24 hours to remove all air from the chambers and canals. Air and water can also be removed by 2-3 lots of absolute alcohol, each of 24 hours. That all air has been removed can be determined under the microscope with transmitted light. The air- and water-free fossils are then placed in anhydrous ether-alcohol for 6-24 hours. Small foraminifera etc. are most easily kept 6-7 togethed in a corked 2-3 ml. tube; larger fossils, eg. bryozoa, individually in 5-6 ml. tubes.

When the fossils have remained long enough in the ether-alcohol they are poured into a not too flat watchglass of 6-10 cm. diameter. Most of the ether-alcohol is removed with filter-paper, without, however, allowing the fossils to become dry, and the watchglass is filled with an approximately 2 % solution of celloidin in ether-alcohol. If there are several fossils they are spaced about 5 mm. apart, and the watchglass then placed in a glass dish whose lid is vaselined. Small foraminifera etc. must remain thus for at least 48 hours, larger ones up to 14 days as the celloidin solution diffuses very slowly.

The thickness of the layer of celloidin over the highest part of the fossil must be at least four times the height of this above the bottom. Finally the celloidin solution is evaporated. This cannot take place too slowly, and a layer of celloidin solution 2 mm. thick must take 3 days to evaporate. One can most easily evaporate the solution by inserting a piece of thin paper under one side of the lid so that there is a small gap in the vaseline; thereafter the whole is allowed to stand until the celloidin is firm, and has assumed the consistence of soft boiled cartilage, or boiled carrots. Evaporation

is terminated by pouring chloroform into the glass dish until it covers the watchglass. (Take care that no vaseline is dissolved in the chloroform!). After a couple of hours the celloidin is cut, under the chloroform, with a thin sharp knife (a razor blade is excellent) so that each fossil lies in a little block with 1-2 mm. celloidin about it. The blocks remain in the chloroform until, in the course of a day, the celloidin is hardened. The blocks may then be stored without limit in 70 % alcohol, but must never become dry, for they dry to a horny consistence and are irretrievably damaged.

In most cases one will, however, at once proceed with the preparation, and begin to remove the fossils themselves.

First the carbonates are dissolved in hydrochloric acid. This is done by placing the celloidin blocks with fossils, after they have been kept for 24 hours in 70 % alcohol to remove the chloroform, in a bath of 1 % hydrochloric acid, 70 % alcohol and 29 % water until all carbonates are dissolved. Whether all carbonates are dissolved can, in the case of calcareous tests, be determined under the microscope as the block will be quite transparent and colourless in transmitted light. The place of the fossil will be shown only by the different light refractions in the celloidin and in the fluid in the cavities where there previously were carbonates. If all carbonates are dissolved the celloidin is rinsed by passing it through 4 lots of 70 % alcohol (6 hours in each) with about 1 % of lithium carbonate added to the second. These blocks are then ready for impregnation.

Whether the carbonates in the arenaceous foraminifera are dissolved cannot be determined, and it must therefore be recommended that they remain for a week in the bath to be quite sure that none remains, after which they are placed in two lots of 70 % alcohol, 6 hours in each.

The silica must now be dissolved in hydrofluoric acid. For this purpose one prepares a bath containing 10 % HF, 70 % alcohol and 20 % water (one part of 40 % HF and three parts of 96 % alcohol). (Take care! Hydrofluoric acid, both in liquid and vapour, strongly attacks skin and mucous membranes.) This bath, which also attacks glass, porcelain etc. must be contained in a platinum crucible with lid, or in lieu thereof in a porcelain dish thickly coated inside with paraffin, and covered with a similarly coated watchglass. It is recommended that the hydrofluoric acid bath be placed in the open air, but so that rain cannot gain entry.

In this bath are placed the celloidin blocks with remains of arenaceous foraminifera, and after at most a week all the silica is usually dissolved. While the blocks are in the bath one can follow the process under the microscope, so long as one takes care not to dip the objective in the liquid, and protects it from the vapour with a piece of cellophane which is destroyed immediately afterwards. Small amounts of dirt and iron compounds from the sand grains will, however, prevent the arenaceous foraminifera from "disappearing" as completely as those with calcareous tests, instead they remain yellowish and cloudy in the celloidin blocks. If the silica is dissolved the celloidin blocks are rinsed in a little 70 % alcohol which is immediately thrown away. The blocks are then taken through four lots of 70 % alcohol, six hours in each, the first being in a wax protected dish, and the second having a little lithium carbonate added. These blocks are then also ready for impregnation.

The blocks are transferred to paraffin through a series of intermediates, and to save time it is satisfactory to do this at an elevated temperature. One can satisfactorily use an embedding oven at 60° C. The celloidin blocks are taken from 70 % alcohol through two lots of 80 % alcohol, each for one hour at 60° C, and thereafter, also at 60° C, through three baths of terpineol of increasing strength. Namely, I, 25 % terpineol, 60 % alcohol and 15 % water, for 12-24 hours; II, 50 % terpineol, 40 % alcohol and 10 % water, for 12-24 hours; III, pure terpineol for 24-48 hours. The celloidin blocks hereby become golden and glass clear, but when they thereafter—still at 60°—are put into pure benzene they gradually assume a milky appearance; this being replaced along the edges by a clear area which progresses inwards, eliminating the milkiness in the course of 2-12 hours. Then the celloidin blocks are put into a new lot of pure benzene, for 4-8 hours, and thereafter into the first lot of melted paraffin (melting point about 56° C). Should the milkiness not disappear there is no alternative but to begin again from the beginning with 80 % alcohol.

It would be best if no liquid were carried by the block from one bath to the next, but as the celloidin may at no time become dry this is an ideal, and as such is unattainable. If one uses very soft pointed forceps one can carefully take the celloidin block, which is very susceptible to pressure, out of the one bath and, after drying off the hanging drop on a filter paper, drop it into the next bath without its carrying much liquid with it.

These seven baths, which must be maintained at 60° C, can readily be accommodated in corked flat-bottomed glass tubes, each of 10-20 ml. capacity, standing in suitable holes bored into a piece of wood; so that all can be taken out of, and put into the thermostat at the same time.

The celloidin blocks are taken direct from the benzene into a watchglass, or flat glass dish, which has previously been filled with so much melted paraffin that the blocks become adequately covered. It can be recommended that the fresh paraffin be divided between two dishes for impregnation. In the first dish is dissolved as much dry, powdered Sudan III as possible, whereby the paraffin assumes a clear red colour. When the celloidin is placed in the coloured paraffin it will absorb the stain and become deep red. The celloidin blocks remain in the first lot of paraffin at 60° C for 24-48 hours, after which one carefully picks them up with warm forceps and lets them fall into the second lot of paraffin where they remain for 12-24 hours at 60° C, after which they are ready for embedding. In preparation for embedding one should smear the inside of the second vessel with glycerine before filling it with paraffin, so that this will "slip" easily. Immediately before embedding, the celloidin blocks are placed, with the aid of a warm needle, in the right position with about 1 cm. between them, and so that that surface which is first to be cut is at the bottom; it is usually that which was downwards during celloidin impregnation. The actual embedding takes place by carefully floating the watchglass, or shallow dish containing the ordered celloidin blocks in the second lot of paraffin, on cold water, and, as soon as a skin has formed over the paraffin, submerging the whole in the cold water. If the first lot of paraffin was coloured the celloidin blocks will now be dark red, and the solidified paraffin pale pink. Orientation will be much eased if one cuts the paraffin, as soon as it is firm but before it is hard, into small pieces, each containing a celloidin block with 2-3 mm paraffin on all sides; allowing the blocks to remain attached to the watchglass or dish. One allows the whole to remain in cold water until the paraffin has become hard; the blocks can then easily be taken off the glass if they have not already freed themselves, and risen to the surface. The small celloidin-paraffin blocks can be stored dry without limit.

When the celloidin-paraffin blocks are to be cut they are most easily attached to a wooden block which has been warmed and

dipped in melted paraffin, by placing them upon this and passing a warm spatula between them and the wooden block.

The blocks are cut on a good sliding microtome with obliquely placed blade (angle between edge and cutting direction 30°-45°). It is absolutely necessary that the blade is newly ground and unchipped. The so-called "b" and "c" blades are best. The section thickness can be varied between 5 and 20 μ. So that the sections will not roll up during cutting, which must take place rather slowly, but steadily, one should rest a fine brush moistened with 70 % alcohol on the leading surface of the section, with the hairs directed towards the knife's edge.

If the celloidin block is stained the celloidin in the section will be light red, and the paraffin white. One can thereby—in many cases macroscopically—observe how far one has reached in the sectioning of the object, since the fossil itself is now replaced by paraffin. The sections are placed in strictly serial order as they are cut, and when all are cut they are stretched, and affixed one by one to slides.

Since celloidin only with difficulty adheres to glass, and as it is important that all sections are included, one must use a strong, and sure adhesive, for this the use of egg-albumin-gelatine is recommended (see p. 24). One takes a 0,5-1,0 % solution of this in a flat glass dish (petri dish), which is placed on a tripod over a eletric bulb, so that dish and solution are both illuminated and warmed. When the solution is at 36°-40° C the sections are laid, with the aid of a brush, upon the surface of the liquid, where they stretch, and become glossy as the paraffin softens. The sections are picked up on a slide and placed in their final position with the aid of a brush or a needle.

If one has kept the sections in 70 % alcohol after sectioning stretching will be extremely intense, and even somewhat rolled up sections will possibly flatten out again.

When the desired number of sections are placed on the slide this is laid, with sections downward, on a piece of smooth moistened filter paper on a glass plate, and pressed lightly (200-300 gm) against the filter paper; partly to be sure that the sections adhere firmly, and partly to remove excess liquid. It is necessary each time to use a fresh portion of the filter paper.

The egg-albumin-gelatine is hardened by placing the slide, with section, in the vapour of 40 % formalin in a closed vessel for one

hour, and thereafter submerging it for 10 mins. in 10 % formol., after which it is carefully dipped in distilled water and dried for 24 hours in a thermostat at 40° C.

In order to see all the details in the sections it will be necessary to remove the paraffin. This is done by taking the slide through two lots of xylene, and two lots of terpineol, about 10 mins. in each.

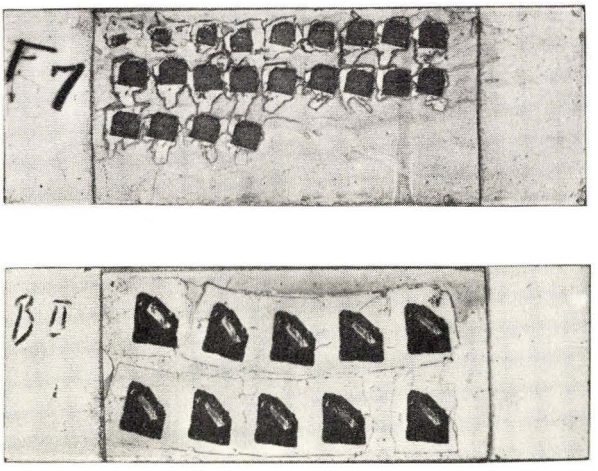

Fig. 1. Two completed preparations of serial sections. Above, of a foraminifer, the sections from No. 2 to No. 22 compose the complete series. Below, part of a series of 48 sections of a bryozoan cut on a serial microtome. The shape of the sections, a rectangle with truncated corner, makes it easy to orientate them similarly when mounting. The rims at some distance from the sections are stained egg-albumin-gelatine, and mark the outline of that part of the original section composed of paraffin, now removed.

This removes the Sudan III from the celloidin, and the sections must therefore be restained.

There are many stains which colour celloidin, but, most are extremely easily washed out again. One of the most stable stains is HANSEN's Haematoxylin (see p. 25) which gives a good reddish-purple colour, but is rather liable to become discoloured. The slides are taken from terpineol through 80 % alcohol to distilled water, and from this into 1 % HANSEN's Haematoxylin. They must remain in this for at least three hours, obliquely placed with the sections downward, so that they are not contaminated by possibly precipitated grains. When the sections are sufficiently stained—they must be darker than the desired final result—they are rinsed for a couple

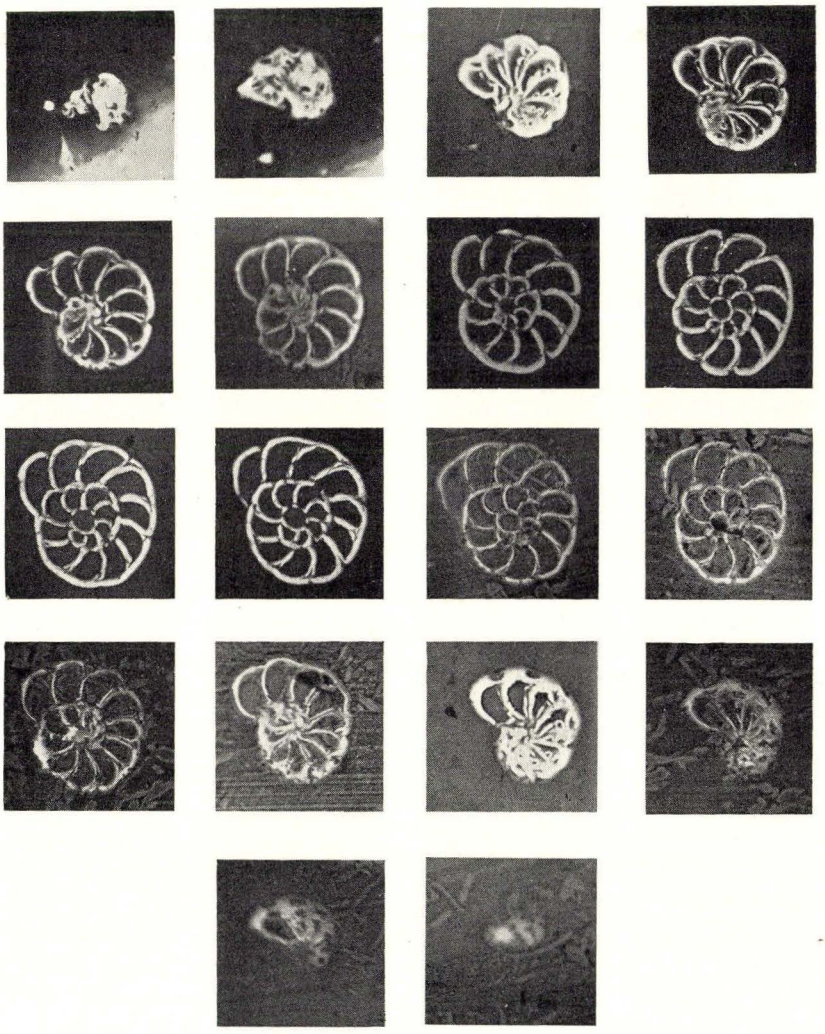

Fig. 2. Complete series of celloidin-paraffin sections of a forminifer with a calcareous test (*Elphidium bartletti* CUSHM.). The "algal" pattern in the celloidin of some of the sections is caused by too great a pressure on uneven filter paper (p. 20). (18 sections of 10 μ thickness. Stain: Mallory. × 52,5 Untouched photograph, J. B.-S.).

of minutes in each of at least two lots of distilled water, or running tapwater—possibly with some carbonate added if one desires a bluer tint—for half an hour, and thereafter some minutes in 80 $^0/_0$

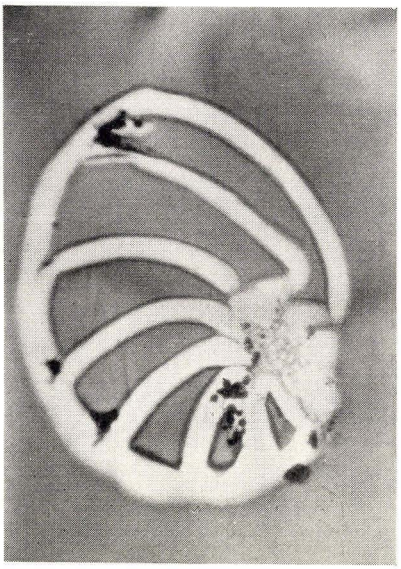

Fig. 3.

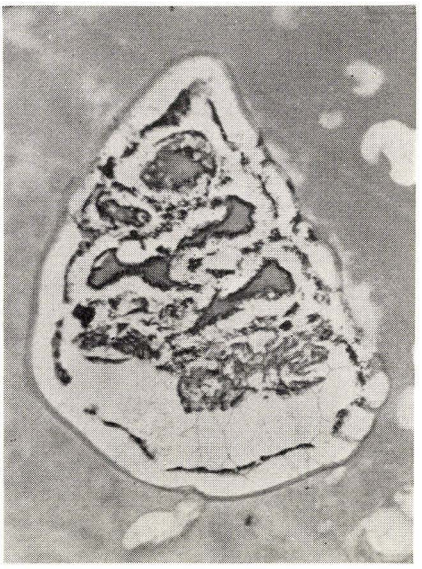

Fig. 4.

Fig. 3. Celloidin-paraffin section of a foraminifer with a calcarecus test (*Nonion dingdeni* CUSHM.) Tangential section parallel to the median plane. The fine canal system in the umbilical region is plainly visible. The double outline of the chambers in places is due to the fact that their limits are not at right angles with the plane of the section. The black spots are pyrites which it has not been attempted to remove; one can appreciate the disturbance which grains of pyrites cause in the section. (The amount of pyrites in this case is about the maximum that can be removed by the method described on p. 16). (Section 10 μ thick. Stain: Mallory, × 105. Untouched photograph, J. B.-S.)

Fig. 4. Celloidin-paraffin section of an arenaceous foraminifer (*Textularia* sp.). The insides of the chambers, and parts of the canal system are lined with small grains, which have not been dissolved. There are also individual undissolved grains scattered in the shell material. (A clear separation is not possible without staining). Note the extremely fine canals in the lowest part of the test. (Section 10 μ thick. Stain: Hansen's Haematoxylin. × 105. Untouched photograph. J. B-S.).

alcohol. The slides are then carefully dried on a piece of smooth damp filter-paper, and placed in terpineol (at room temperature). The sections must be taken through at least two lots of terpineol, 30 mins. in each, and after being rinsed in xylene, must remain in a bath of pure xylene for 10-20 mins. The sections must now be quite clear, and nearly invisible against a black background. If they have milky patches, it is because they contain water, and they must then

be taken back through terpineol to 80 % alcohol, and thereafter, as before, through terpineol to xylene.

If the sections are clear the slides are placed, still wet with xylene, on the table, syruppy dammar-xylene dropped on, and a clean cover glass laid over, and pressed steadily; the whole is then dried at about 40°C. for 48 hours, and the sections are then ready for labelling etc.

MALLORY's stain is a better, but slightly more difficult stain which quickly, in the course of 15-20 mins., gives an intense ultramarine blue colouration. With this stain the slides are rinsed in distilled water after staining, and after being thoroughly dried on both sides are taken direct to terpineol, after which one continues as in haematoxylin staining. This method demands some practice for success, but the result is very attractive.

The celloidin-paraffin technique can also be used for recent forms where the actual animal is still in the test. In this case one must not, of course, dry the animals in the thermostat before the celloi-impregnation, but take them slowly through increasing concentrations of alcohol (30 %–50 %–70 %–85 %–96 %–99 %) to absolute alcohol. So that the protoplasm will show more pronouncedly they should not be so deeply stained. The celloidin can finally be removed with clove-oil or ether-alcohol, so that only the protoplasm remains. (BÖDECKER's Celloidin Section Technique, p. 14).

Appendix.

Anhydrous absolute alcohol, ether-alcohol and terpineol are maintained by keeping some anhydrous copper sulphate on the bottoms of the bottles in which the liquids are kept.

Celloidin, 2 %, is prepared thus: The celloidin, which is generally bought in sheets, is shreded, and dried for a week at 40°C. One gramme of the resulting coarse celloidin powder is weighed out for each 50 gm of ether-alcohol and the two mixed in a bottle with a well fitting cork. The mixture is shaken a couple of times a day, and after a week all the celloidin is dissolved and ready for use.

Egg-albumin-gelatine (after OLT, 1906) is made from 10 gm of purest gelatine dissolved in 100 ml. distilled water on a water-bath, to which the white of a fresh egg is added little by little

while stirring. The whole is boiled for 30 mins. on a water bath with continuous stirring, and is filtered hot. To prevent the growth of moulds, a couple of crystals of thymol dissolved in a couple of mls. distilled water are added last. One assumes this solution to have a strength of 10 %.

Ether-alcohol is made of equal parts of ether and abs. alcohol.

HANSEN's Haematoxylin is prepared (from F. C. C. HANSEN, 1895), thus:

a. 1 gm. haematoxylin is dissolved in 10 ml. absolute alcohol.
b. 20 gm. potassium alum are dissolved in 200 ml. distilled water, and filtered after cooling.
c. 1.0 gm. potassium permanganate is dissolved in 16 ml. distilled water.

The following day a. and b. are mixed, and exactly 3 ml. of c. are added slowly with stirring. The mixture is boiled for 30-60 mins. (not longer) with stirring, and is quickly cooled and filtered. The colour remains fast for a long time.

Hydrofluoric acid which has to be kept indoors for a longer period must have an air-tight paraffin seal, so that the vapour does not escape and cause damage.

MALLORY's Stain is made from:

0.5 gm. water-soluble aniline blue (GRÜBLER)
2 gm. Orange G (GRÜBLER)
2 gm. Oxalic acid dissolved in 100 ml. distilled water

The solution is boiled, cooled and filtered. It does not deteriorate for a long while. Aniline blue, which makes the most important contribution to the deep blue colour does not, strangely enough, work well in pure solution.

The Strengths of solutions are expressed as percentages; by which meant the percentage of pure substance: eg. 10 % formol is made from three parts of distilled water and one part of concentrated formol (formalin) as it is bought at about 40 % (not 9 parts water and 1 part of bought, concentrated formalin—this would give a 4 % solution).

Used Terpineol can be recovered from water, alcohol and benzene by warming to 110° C (But note that benzene vapour forms explosive mixtures with air).

Washing of tests may be caried out in tapwater, but the last rinse before drying should always be distilled water. Clay or other difficultly removable matter may, however, be removed by cooking the tests, one or more times, in a 5 % aqueous solution of sodium carbonate, or, better, in 10 % hydrogen peroxide before washing.

List of technical words.

English	Danish	French	German	Formulae
abs. alcohol	absolut alkohol	alcool absolu	absoluter Alkohol	C_2H_5OH, 99,9 %
ammonia	ammoniak	amoniaque	Ammonium	NH_3 sol. in H_2O max. ca. 24 %
alcohol	alkohol	alcool	Alkohol	C_2H_5OH sol. in H_2O 0—100 %
Anilin blue soluble in water (Grübler)	(Trade name for a mixture of NH_4^+, Na^+ and Ca^{++}-salts of triphenylrosaline sulphonic acids)			
benzene	benzol	benzène	Benzol	C_6H_6
canada balsam	kanadabalsam	baume de canada	canadischer Balsam	
celloidin	celloidin	celloidine	Celloïdin	
chloroform	kloroform	chloroforme	Chloroform	$CHCl_3$
copper sulphate	kuprisulfat	sulfate de cuivre	Kupfersulfate	$CuSO_4$
dammar	damarharpix	résine de dammar	Damarharz	
egg albumin	æggehvide	blanc des oeufs	Eiweiss	
ether	æter	éther	Äther	$C_2H_5O \cdot C_2H_5$
formol	formol	aldéhyde formique	Formol	HCHO sol. in H_2O max. ca. 40 %
gelatine	gelatine	gelatine	Gelatine	
hematoxylin	hæmatoxylin	hématoxyline	Hämatoxylin	
hydrochloric acid	saltsyre	acide chlorhydrique	Chlorwasserstoff	HCL sol. in H_2O max. ca. 36 %
hydrogen peroxide	brintoverilte	eau oxygénée	Wasserstoff peroxyd	H_2O_2 sol. in H_2O max. ca. 30 %
hydrofluoric acid.	flussyre	acide fluorhydrique	Fluorwasserstoff	HF sol. in H_2O max. ca. 40 %

English	Danish	French	German	Formulae
lithium carbonate	lithiumkarbonat	carbonat de lithium	kohlensaures Litium	Li_2CO_3
oil of cloves	nellikeolie	essence de girofle	Nelken-öl	
Orange G. (Grübler)	(Na-salt of benzene-azo-β-naphthol-disulphonic acid)			
oxalic acid	oxalsyre	acide oxalique	Kleesäure	$(CO_2H)_2$
paraffin	paraffin	paraffine	Paraffin	
potassium alum	kalialun	alun potassique	Kaliumaluminiumsulfat	$AlK(SO_4)_2$
potassium carbonate	kaliumkarbonat	carbonat de potasse	kohlensaures Kalium	K_2CO_3
potassium permanganate	kaliumpermanganat	permanganat potassique	Kalium permanganat	$KMnO_4$
pyrites	svovlkis	pyrite de fer	Schwefelkies	
Sudan III	(benzene-azo-benzene-azo-β-naphtol)			
terpineol	terpineol	terpineol	Terpineol	
vaseline (petrolatum)	vaseline	vaseline	Vaseline	
xylene	xylol	xyléne	Xylol	C_8H_{10}

Dansk resumé.

Efter en kortfattet oversigt over det praktiske foramineferstudiums historie, gennemgås de metoder, der gennem tiderne har været brugt for at studere skallernes indre bygning, idet hver metode er gengivet efter den originale beskrivelse. Derefter forklares i enkeltheder en ny metode til fremstilling af varige seriesnit mellem 5 og 20 μ af kalkskallede og aggluthinerende foraminiferer og andre småfossiler. Principet i denne metode er, at først indstøbes fossilen i celloidin, derefter opløses selve fossilen, og det derved fremkomne hulrum udfyldes med paraffin, der ligeledes omstøber hele celloidinblokken. Efter skæring på mikrotom opklæbes snittene, parafinen fjernes og celloidinen farves, hvorved selve fossilmaterialet kommer til at vise sig hvidt på farvet baggrund, ligesom alle porer og kanaler viser sig farvede. Metoden muliggør således flere rekonstruktioner af kanalsystem o.s.v. af samme individ.

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Index.

	page
Acid celloidin, Bödeker	14
adhesion of sections,	
author's method	20
Bödeker's method	15
alcoholic fuschsin, staining	13
anhydration of liquids	25
arenaceous foraminifera,	
serial sections	15
Lecrist	12
artifical stone casts	11—13
author's serial section technique	15
Batsch, A. J. G. C.	10
Beccari	9
Beissel, P.	11
Beissel's stone-cast method	13
Bianchi, G. P. S.	9
Bödeker, C. F.	11
» 's celloidin sections	14
» 's serial section machine	11
Brotzen, F.	11
bryozoa, serial sections	15
bryozoa, stone casts	13
canada balsam impregnation, Hofker	15
canada balsam-shellac, Rosenbusch	13
carmine, filling of tests	11
Carpenter, W. B.	11
Carter	11
casts of foraminiferal, and other tests	11—13
celloidin, preparation	24
celloidin sections with protoplasm	14
» » of fossils	15
control of pyrites	16
cutting of celloidin-parafin blocks	20
solution of tests	17
echinoderm spines, serial sections	15
egg-albumin-gelatine, preparation	24
egg-albumin-glycerine	15
Ehrenburg	11
etching ground sections	12
ether-alcohol, preparation	25
formulae	27
fuchsin, staining	13
grinding	9—10—12
grinding and staining method	11
Hansen's Haematoxylin, preparation	25
» » , staining	21
Hofker, J.	12
» 's canada balsam impregnation	15
iron compounds in pores	12
Johnston, Christopher	11
» 's grinding method	12
Lecrist	12
Linné, K. v. (Linneus, C.)	9
Mallory's stain, preparation	25
» » , staining	24
Mandl, Louis	11
Martini, F. H. W.	9
machine for serial sections	11
models of foraminifera, d'Orbigny	11
Möbius, K.	11
» 's stained, ground-sections	13
mounting after Hansen's Haematoxylin	22
» » Mallory's stain	24
natural stone casts	11
d'Orbigny, A. D.	10
Owen, Richard	11
paraffin embedding, author's	19
» » , Bödeker's	14
Plancus, Janus	9
pyrites	16
removal of air and water	16
Rosenbusch	12

	page
RUTTEN, M. G.	12
shellac	13
sections	11—13—14—15
serial sections of fossils	15
» » » recent animals	14
staining celloidin-paraffin blocks	19
» ground sections	11
» with HANSEN's Haematoxylin	21
staining with MALLORY's stain	24
strength of solutions	25
Sudan III, staining	19
thermostats	18—19
terpineol cleaning of	25
» , use of	18
water-glass	13
water, removal of	16
WILLIAMSON, W. C.	10

Outline of the Two Serial-Section Techniques.

Tests with soft parts

30–50–70–85–96–99 % alcohol, at least two hrs. in each

Empty tests

cleaning

dry at 100° C. for 24 hrs.

absolute alcohol

Bödeker's method, only for recent forms with calc. tests.

Methyl alc., 1–2 hrs.

20 cc. acid celloidin, 1–8 days (without disturbance)

evaporation of celloidin

cutting out

70 % alc., 1–2 hrs.

40 % alc., 1–2 hrs.

aqueous alum, 24 hrs.

running water, 6–8 hrs.

30–50–70–85–95 % alc., each 1–2 hrs.

aniline, 6–12 hrs.

aniline-chloroform, 3–6 hrs.

chloroform, 6–12 hrs.

weak soln. paraffin in chloroform, 3–6 hrs. at 20° C.

strong soln. paraffin in chloroform, 3–6 hrs. at 38° C.

pure melted paraffin, 2 hrs at 60° C.

embedding and trimming

sectioning

adhesion of sections to slide

xylene, 3 mins., to dissolve paraffin

ether-alc. to dissolve celloidin

absolute alcohol

96–85–70–50–30 % alc., each 1 min.

distilled water, 1 min.

staining and mounting.

Author's method: for all animals and empty tests.

Anhydrous ether-alc., 6–24 hrs.

2 % celloidin, 2–14 days

evaporation of celloidin, at least three days

hardening in chloroform, and cutting out (24 hrs.)

70 % alc., 24 hrs.

1 % HCl + 70 % alc. + 29 % H_2O

Arenaceous tests remain 8 days

70 % alc., 2 × 6 hrs.

10 % HF + 70 % alc. + 20 % H_2O for 8 days.

rinse in 70 % alc.

Calcareous tests remain in this for one to several days.

70 % alc. + Li_2CO_3, 6 hrs.

70 % alc., 3 × 6 hrs.

80 % alc., 2 × 1 hr. at 60° C.

25 % terpineol + 60 % alc. + 15 % water, 12–24 hrs. at 60° C.

50 % terpineol + 40 % alc. + 10 % water, 12–24 hrs. at 60° C.

100 % terpineol, 12–24 hrs. at 60° C.

pure benzene, 2–12 hrs. at 60° C.

pure benzene, 4–8 hrs. at 60° C.

paraffin I (with Sudan III), 24–48 hrs. at 60° C.

paraffin II, 12–24 hrs. at 60° C.

embedding and trimming

sectioning

stretching and softening of the sections with egg-albumin gelatine

pressing on damp filter-paper

hardening in formalin vapour, 1 hr.

hardening in 10 % formalin (liquid) 10 mins.

drying for 24 hrs. at 40° C.

removal of paraffin: 2 × xylene, and 2 × terpineol

80 % alc., 15–60 mins.

distilled water, 5 mins.

1 % Hansen's haematoxylin, 3–24 hrs.

running water, 30 mins.

80 % alc., 2 × 10 mins.

Mallory's stain, 15-30 mins.

distilled water, 10 mins.

dry on damp filter-paper

pure terpineol, 2 × 30 mins.

xylene rinse

xylene, 10-20 mins.

mount in dammar-xylene (or canada balsam).