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BIG THREE IN SNOWMAKING. -- Perfectly preserved snowflakes encased in plastic and mounted on glass are studied by General Electric's trio of weather scientists, Dr. Bernard Vonnegut, Dr. Irving Langmuir, and Dr. Vincent J. Schaefer. The three are responsible for man's newfound ability to produce snow and rain from clouds.

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ON THE NATURAL COLORING MATTER, *BRAZILIN*, AND ITS USE IN MICROSCOPICAL TECHNIQUE.

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I. HISTORICAL REVIEW.

Among the natural coloring matters, brazilin is the only one dating from the pre-Columbian era to survive the competition of the synthetic dyestuffs in microscopical technique, and although it has had staunch and distinguished advocates (Eisen, Schaudinn, Hickson, Champy, Belling), it is known to most of today's microscopists, if at all, vaguely as a red homologue of hematoxylin. Because it has had a particularly interesting history and because for a few specific purposes it offers advantages over other stains known to the writer, the following account is offered.

While the dyestuff is not so old in commerce as the reds from madder (known in Egypt even in pre-dynastic times), during the Middle Ages a red dyewood coloring matter was imported into Europe from southern Asia under the varying names, "brasilium", "bresillum", and "brezellem" (Oxford English Dictionary, I:1066). The name is of uncertain origin, several possible etymologies having been suggested, among them that it is a corruption of an Asiatic word, presumably now lost, for the redwood. According to Leggitt (1944, p. 50), the dyestuff appears as *verzino*, the usual Italian designation, in the taxlists of Ferrara as early as 1193 and of Barcelona, in Moslem Spain, in 1280. Sarton (II:1042) cites the use of brazilwood to make rose-colored letters in manuscripts as described by Abraham Ibn Hayyim working at Loulé, Portugal, in 1262. Marco Polo (c. 1254-1324) reported the occurrence of brazil-wood forests in Sumatra, Siam, the Nicobars, Ceylon and along the Malabar coast of India and even tried (the seeds did not thrive) to grow brazil at Venice. He distinguished among the qualities of wood from the different sources. At this same period, according to Yule (1875), brazil-wood was a royal gift from Siam to China in customary interchanges between the two courts.

Most specific information available on the dyewood commerce of the early Renaissance is that in the veritable handbook of international trade *(Libro di divisamenti paesi e di misure di mercatantie)* compiled by the Florentine, Francesco Pegolotti, traveller and merchant of the international banking house of Bardi, about 1340 (Yule, Sarton). Pegolotti recognized several types of brazil of which *verzino colomi* or *colombino* was definitely that of Quilon on the Malabar coast (Yule), the kind held in highest esteem (i.e. commanding the highest price). *Verzino Ameri* is taken by Yule to have been of Lambri in northwest Sumatra, a grade of good quality, while *verzino Seni* (possibly for Sini) may have been that brought by Chinese merchants from Siam as far as India, a grade costing about a third as much as the Malabar variety. The fascinating *Ribla (Journey)* transcribed in 1356 by Ibn Juzayy from the verbal account of Ibn Battuta, the greatest traveler, "not excepting Marco Polo, in medieval times" (Sarton III:1614), contains a description of brazil-wood on the Malabar coast paralleling and confirming Polo's earlier account.

In England, whose textile and dyeing industries lagged behind those of the Italian city-states, of France and of the Low Countries, the red dyewood had a vernacular name by the fourteenth century as Chaucer's Nun's Priest (*Canterbury Tales*, ca. 1386) uses the phrase, "His colour for to dyghen with brasile". This "brasile" (a baker's dozen variant spellings are provided by the *Oxford English Dictionary)* or brazil-wood was the product of a leguminous tree, *Caesalpinia*, and probably mainly the Sappanwood, *C. Sappan*, although other caesalpinias of the Asiatic tropics may have been included. The adventurous Venetians were the major importers of the dyestuff across the land routes from India, supplying the dyers of the rest of Europe.

With the discovery by the Portuguese of an all-water route to India, the center of gravity of the spice and dyewood trade shifted from Italy to the Iberian peninsula. Soon thereafter a vast new supply of brazil-wood was found by the same Lusitanians in the Western Hemisphere as recounted proudly by Camoens (1572) in his epic of the Portuguese people, *Os Lusiads:*

"Mas ca onde mais se alarga, ali tereis
Parte tambem, co'o pau vermelho nota
De Santa Cruz o nome lhe poreis;"

"But where the land spreads broadest ye shall claim
The part that for its red wood is renowned
Of Santa Cruz ye shall bestow the name.
(Aubertin translation of 1884)

This Santa Cruz "co'o pau vermehlo", with the red dyewood tree and for it called alternatively Terra de Brasil, became quickly Brasil (on which the historian De Barros commented sourly "as if the name of a wood for coloring cloth were of more moment than that of the Wood which imbues the Sacrements with the tincture of Salvation" -- and attributed the change "to the suggestion of the Evil One" (Yule, *op. cit.*, p. 368). The New World yielded also Campeachy wood or logwood, the source of hematoxylin. It is curious that both brazil and logwood suffered marked disfavor in England. As early as the thirteenth century the bye-

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laws of the Painters' Guild of London forbade painting on gold or silver except with fine (mineral) colors " e nient de brasil, ne de inde de Baldas, ne de nul autre mauveise couleur" (Yule, op. cit., p. 371). Act 24, 1532-1533, of Henry VIII states, "Diers.....haue vsed deceyuable waies in dyeing with brasell and such other lyke subtilties" ("deceivable" here with a now defunct meaning "having the habit of deceiving"), while, Leggett (1944) tells us of logwood, "In 1580, an Act of Parliament forbade it to be used for dyeing, and large quantities thereof were burned"

Biologically decoctions of brazilwoods appear to have been used earliest by Reichel (1752, 1758) in his investigations of the vessels of plants. After boiling scrapings of Pernambuco wood, he dipped plant stems (*Phaseolus, Lupinus*) into the cooled brew and, because the colored fluid passed along the spiral vessels, he concluded that these carry sap and not air as maintained earlier by Malpighi. Reichel cut both cross and longitudinal sections of the stained stems. A little later Hedwig (1782) applied the method to tissues of squash and Mayer (1793) did further work on plant vessels with Pernambuco and brazil-wood decoctions. Lewis (1942) remarks, "It was inevitable that logwood *(Haematoxylon)* should soon be used in these injections" and describes the first use of that tincture by a biologist, Knight (1803, 1808), in the same manner as were the brazil decoctions on similar plant materials.

Saefftigen (1884) of Heidelberg, a student of Otto Bütschli, was at least among the earlier workers using brazilin on animal tissues having employed unspecified brazilin and hematoxylin methods on sections of thorny-headed worms (Acanthocephala). Both Breglia and Flechsig in 1889 published neurological methods employing redwood extracts. Breglia stained sectioned pieces of central nervous system (mammalian) fixed in Müller's or Erlitzki's fluid and mordanted with lead, iron or copper salts in various aqueous solutions of Pernambuco wood extract. The extractions, like those he made from logwood for hematoxylin, were carried out with alcohol. Borax and ferrocyanide mixtures were in some cases used with the dyes.

Flechsig combined a Golgi sublimate-dichromate impregnation of human brain with his brazilin stain cutting his sections at 50 microns. For this he made a stock solution of one gram of pure extract of Japanese redwood in ten grams of absolute alcohol. Diluting this with 900 cc. of water and five of a saturated aqueous solution of Na_2SO_4 and tartaric acid, he stained the sections three to eight days at about 35° C. After washing, the sections were treated with potassium permanganate followed by an oxalic acid--potassium sulfite bath as with a Weigert-Pal stain. Rawitz (1895) mentioned the use of an alum brazilin employed as a nuclear stain but gave no particulars. Heimann (1898) used a "Delafield's brazilin" for staining ganglion cells and likewise provided no details about his procedure.

Eisen (1897) proceeded from Böhmer's alum hematoxylin formula apparently being the first to use brazilin in a really effective manner on a variety of objects. He allowed his solutions to ripen for a few weeks until they were a deep, brilliant red with numerous bluish flakes. These flakes, insoluble in water, he considered to be brazilein and these he filtered out, dissolving them in glycerine-alcohol for use. His critical judgement was that "for scientific investigations of (animal) cell structure and cell differentiation both hematoxylin and brazilin have but little use". He considered brazilin a satisfactory stain for classroom or pathological material and a very good dye with no tendency to overstain for plant materials. Of its metachromasy he noted "with some tissues it is a treble stain", e.g. with newt spermatozoa. He used weak solutions of nigrosin or indulin for counterstaining.

Wide use of brazilin began with Eisen, Schaudinn (1900) and Hickson (1901) just as Hickson was impelled to remark about brazil-wood, "of recent years it has been superseded by other colouring substances and practically driven out of the market". Major supplies of brazilin in the last 75 years have come from a few species of *Caesalpinia,* a tropicopolitan genus of leguminous trees, although possibly the same tinctorial principle could be extracted from many species of *Caesalpinia* and the closely related genus *Peltophorum.* These are known as the soluble redwoods or the brazil-woods in contrast to another group of of dyewoods, the insoluble redwoods (barwood, camwood, etc.) which do not yield their color content to aqueous extraction.

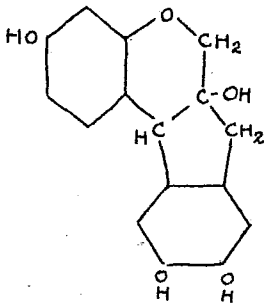
The original "brazil" is very likely *Caesalpinia Sappan* Linnaeus (from Malay "sapang" probably meaning Japan referring to supposed origin) indigenous to the Asiatic tropics from India to Malaya and present in the Philippine Islands at least from prehistoric times. The best of dye sources is Pernambuco wood from *C. crista* Linnaeus of Brazil and Jamaica (although the species is pantropical according to Merrill). Brazil-wood (in the current restricted commercial sense,) *C. brasiliensis* Linnaeus, which also grows in Brazil, is reported to yield about half the amount of pure dye per unit of wood obtainable from Pernambuco Wood. The fact that all three of these trees were known to Linnaeus and that he described and named them in his *Species Plantarum* of 1753 is some indication of their prominence.

Caesalpinia echinata Lam., of South and Central America is often called peachwood and is a dyewood of good yield. While the dyers' cant seems to be a little less than standardized, the foregoing equivalents (scientific and commercial) appear to be generally valid. The writer can give no reasonably precise equivalents for the following: All Souls' Wood, Bahamawood, Bahiawood, Bukkumwood, Jamaicawood, Japanwood, Limawood, Nicaraguawood, and St. Martha wood. They may refer to one or more of the species above or to other Caesalpinias or Peltophorums of minor commercial importance. Brasiletto (braziletto) and sobrazil have been variously applied, but somewhat more con-

sistently to the species of low yield. Hypernic, seemingly confined to American dyers' cant, refers either to a soluble redwood or to raw extract. Dividivi, which may include *Caesalpinia tinctoria,* is referred to by Knecht (1911) as used like Pernambuco wood in textile printing, but the name applies more usually to species employed for their tannins.

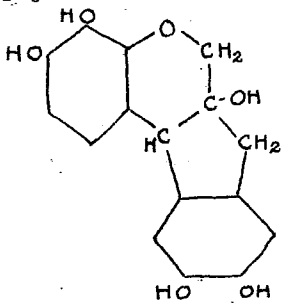
As early as 1808 M. E. Chevreul crystallized pure brazilin from soluble redwood. In the following century particularly through the investigations of English and German chemists the empirical formula of the compound and its probable structural formula were elucidated being respectively $C_{16}H_{14}O_5$ and, according to Pfeiffer,

BRAZILIN



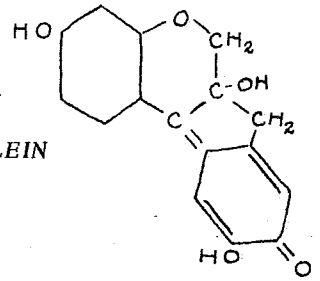
In comparison hematoxylin may be considered a brazilin oxidized one step further to $C_{16}H_{14}O_6$ with the structural formula:

HEMATOXYLIN

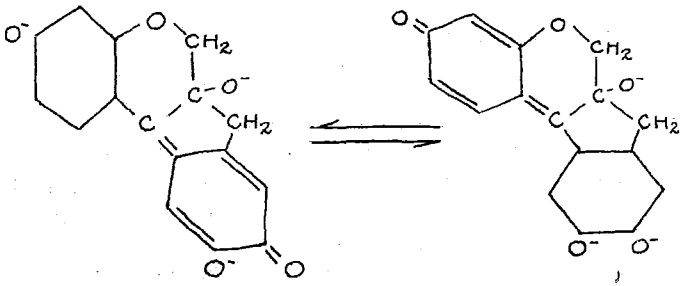


A molecule of brazilin with one and a half molecules of water forms a light red crystal of reported bittersweet taste (the writer's powdered, presumably not hydrated supply, tasted unintentionally, is very bitter and not at all sweet). Brazilin itself is a leuco-compound, its solutions being without color or at least pale in pure form. On oxidation, which proceeds somewhat slowly in absolute alcohol and more rapidly in aqueous solution, two hydrogens are dislodged leaving brazilein, $C_{16}H_{12}O_5$, which is reddish brown in solution precipitating in shiny, silvergray flecks reddish brown with incident light.

BRAZILEIN



Resonance (isorrhopesis), an equipoise between two of the theoretically possible ionized forms, is thought to be responsible for the coloration.



RESONANCE OF TWO IONIZED BRAZILEIN FORMS

Brazilein is, then, a chromophore or color-bearing compound, but it lacks strong auxochrome groups to make it a fast dye. Likewise hematoxylin is oxidizable to a homolog of brazilein, hematein, which is a chromophore. Industrially brazilin is commonly used either in the form of raw decoctions or in purer form with a variety of salts which promote oxidation and form lakes (with the brazilin) which are complete stains (i.e. have color and fastness) the lakes being formed either before dyeing or in the thing to be dyed itself. Formerly brazilin in various forms was widely used in the dyeing of cotton and wool, in printing fabrics, in coloring leather, in the making of wall-paper, and in the compounding of red writing ink. Tin, chromium, aluminium and iron salts were used as mordants. Karrer states, "even today it is still used in cotton printing, and for dyeing cotton which has been mordanted with sumach or tin salt".

Use in microtechnique, although it follows the general lines of industrial methods in some respects, requires a pure product such as is well defined under Colour Index No. 1243 (or Schultz Farbstofftabellen Num. 1375). Biological staining methods employing the purified com-

pound (as opposed to the decoctions of Reichel and others) may be considered as members of groups of varying importance. First of these was the methylene blue-brazilin combination originated by Schaudinn (1900) and employed by him on the shelled rhizopod, *Trichosphaerium* and later by Lücke on the foraminiferan, *Saccammina.* Sections were stained five minutes in saturated aqueous methylene blue and a day in brazilin (solution not specified) and were then differentiated about an hour in 43% ethyl alcohol. By this method nuclei were stained clear red, inclusions blue and plasma pink.

The alcoholic iron brazilin technique of Hickson (1901) is certainly the most influential of brazilin methods yet devised. Hickson mordanted sections from one to three hours in a 1% solution of ferric alum in 70% ethyl alcohol, rinsed in 70% alcohol and stained in 0.5% brazilin in 70% alcohol from three to 16 hours. He differentiated in 70% alcohol, dehydrated, cleared and mounted. Hickson used the stain on a number of animal tissues including those of newt, dog and cat as well as on the suctorian, *Dendrocometes.* He held the advantages of alcoholic iron brazilin to be the avoidance of water and excellent metachromasy.

Hickson's regimen has been used and modified by various botanists. Miss Dale (1903), who used alcoholic iron brazilin on fungi, spoke of its results as "very certain" with no overstaining. Her preparations seemed equally good whether material was stained before or after sectioning. Cejka (1912), after fixation with mercuric chloride solutions followed by iodine treatment, used alcoholic safranin-brazilin stain regressively for a fungus of human hair.

It was on Hickson's stain that Belling (1928) based his very precise iron brazilin method for pollen mother cells. Following a chromic-formol-acetic fixation, pollen mother cells were washed carefully, mordanted in fresh alcoholic ferric alum as much as three days, washed again, and stained for some hours in a well ripened solution of 0.5% brazilin in 70% alcohol. Belling made up his brazilin solution with absolute alcohol, differentiated his materials under the microscope (100-200 diameters magnification), and used only fresh, clear alcoholic ferric alum if destaining other than with alcohol alone was required. These refinements along with the deliberateness of his procedure may account for the fineness of his results. (Conversely, some of the criticism of brazilin for similar cytological work may spring from techniques less exacting). The schedules reported by Webber (1929), Capinpin (1930) and Sax (1931) are acknowledgedly derivative--and only that of Webber differs enough from Belling's to warrant review. Webber fixed anthers only ten minutes in a mixture of ten parts of glacial acetic acid to 25 parts of absolute alcohol, washed in absolute alcohol and moved through a graded series to 60% alcohol from which he changed to an alcoholic ferric alum mordant. He mordanted only an hour and stained the anthers for a like period in 0.5% alcoholic brazilin differentiating in 70% alcohol followed by alcoholic ferric alum. This method, which is markedly

shorter than Belling's, Webber considered good for matured chromosomes, cytokinesis, tetrads and some other structures, but he recommended the original Belling schedules for finer details of the thread stage. Both Belling and Webber recommended use of yellow-green filters for observation of brazilin-stained preparations.

Criticism of the alcholic iron brazilin and iron hematoxylin methods for pollen mother cells (characteristically milder for brazilin, however) has been voiced by Darlington (1933) and Darlington and La Cour (1947) and echoed by Grigg (1946) particularly on the score of density of cytoplasm as an obscuring factor in such preparations. Some precautions against such density will be discussed in part two of this paper.

It is not to be inferred from the foregoing account that the botanists preempted use of the iron brazilin method of Hickson. Other British zoologists employed the technique (*inter alia* Dendy, 1914, working on gametogenesis on the sponge, *Grantia,* at Plymouth); in the United States it appears to have been favored at Harvard (cf. Smallwood, 1904, on snail maturation stages and other workers on coelenterates, trematodes and chicks); in Bohemia at Prague it was employed extensively (*inter alia* by Bilek, 1909, for ascarid cytology; Vejdovsky, 1912, and Vesely, 1913, for insect spermatogenesis). Gutherz (1922) commented favorably on the nuances achievable with the method as well as upon its permanence. These are references encountered in the course of reading and do not constitute a balanced account of the use of alcoholic iron brazilin in zoology.

Champy (1913) showed brazilin to have considerable possibilities for variation as a zoological stain. For rapid work he used it like a Weigert's hematoxylin (but with tones in red) and counterstained with light green. To achieve a preparation resembling somewhat one stained with the safranin and light green of Benda, he would fix in Flemming's solution and dye with ammonium alum brazilin (see Champy's plate II, figures 4, 5, 12). The alum brazilin he made by adding 5% of a saturated alcoholic solution of dye to a hot saturated solution of ammonium alum. With the supernatant portion of the cooled mixture he stained 20 to 25 minutes and counterstained with light green. Most striking was Champy's third method. Sections of amphibian testis fixed in Bouin's fluid he stained in iron hematoxylin and differentiated somewhat more than ordinarily. Then he stained the sections 24 hours in alum brazilin, differentiated slightly with alcohol, finishing with either light green or Congo red. Effects of this multiple stain are shown in Champy's plates VI and VII.

Better known in the United States is Bensley's "Brasilin-Wasserblau" combination (Bensley, 1916; Bensley and Bensley, 1938). Published color plates show effective use of this technique on thyroid and on pancreas. Endocrine tissues preserved in Zenker's or Zenker-formol fixative are stained for one to several hours in freshly prepared phosphotungstic acid - brazilin solution:

Phosphotungstic acid	1 gram
Distilled water	100 cc.
Brazilin	0.05 gram

(ripened with a few drops of barium carbonate filtered hydrogen peroxide or of molybdic acid).

Sections are counterstained in phosphomolybdic-Wasserblau solution (phosphomolybdic acid, 1.0 gram; aniline blue, water soluble, 0.2 gram; water, 100 cc.) one to five minutes, washed rapidly in distilled water, dehydrated in several changes of absolute alcohol, cleared in toluene and covered with mountant. Brief mordanting in fresh stannic chloride solution improves contrast, "but will detract greatly from the transparency and beauty of the preparation". By this method chromatin stains red, secretion antecedent in pale blue droplets; mitochondria, reddish purple; connective tissue, bright blue; and erythrocytes, orange red.

Mawas (1919), utilizing the fact that brazilin by itself is easily removed from tissue while with metals it forms fast lakes, devised a useful histochemical test for iron. Tissues fixed with non-metallic, non-mordanting fluids were imbedded, sectioned, decerated, hydrated and stained with 0.5 to 1.0% aqueous or alcoholic brazilin coloring tissue iron dark brown and chromatin red violet. Chloroform-alcohol would, moreover, extract the dye from the chromatin while the brazilin lakes of the tissue iron were not affected.

O'Leary's brazilin method for myelin sheaths (Cowdry, 1948) proceeds from nervous tissue fixed and mordanted with Müller's fluid up to a day and sectioned in either paraffin or celloidin. The staining fluid, compounded of 10 cc. of well ripened 10% brazilin (Grübler) in absolute alcohol and five drops of glacial acetic acid in 100 cc. of distilled water is applied for an unspecified period. Sections are washed in distilled water and differentiated briefly (up to five minutes) in 0.25% potassium permanganate. When gray matter appears pink and white matter is brilliant red, differentiation is complete. Excessive action of the permanganate is checked with Weil's solution (weak oxalic acid-sodium bisulfite solution). Incomplete differentiation can be remedied by reapplication of permanganate followed again by Weil's solution. Further treatment depends on the mountant used.

There remain a few miscellaneous applications of brazilin. Bensley and Bensley (*op. cit.*, p. 107) recommended brazilin as a background stain for muchematein preparations. Reichert (1909) used aqueous brazilin (or hematoxylin) with added hydrogen peroxide for demonstration of flagella of bacteria. Mencl (1911) used a conventional Hickson alcoholic iron brazilin in his study of the "nuclear equivalents" of *Azotobacter* and for Guarnieri bodies, remarking its superiority over Heidenhain's hematoxylin for these objects. Maire and Tison (1909) are the only workers, to the writer's knowledge, to use a brazilin-eosin combination. These investigators employed an iron brazilin with eosin (after fixation in Maire's aqueous picro-formol preservative) on the plasmodiophoran, *Sorosphaera veronicae*.

Yamaha (1937) used a number of dyes, among them brazilin and hematoxylin, in paraffin oil as vital stains for algae, apparently in the case of these two with unsatisfactory results.

In summary extracts of brazil-wood have been used for dyeing fabrics from medieval times. Soluble redwood extracts have been used in biology since the eighteenth century and from the latter part of the last century various techniques employing pure brazilin have been applied. Of the various combinations devised the most generally valuable has been the alcoholic ferric alum followed by alcoholic brazilin of Hickson but various mordants other than ferric alum have been used. Methylene blue, Wasserblau, light green, safranin, eosin and Congo red have been used as counterstains. It is reported to be effective after a variety of fixatives including the picro-formol and picro-formol acetic, craf, osmium tetroxide containing and mercury-dichromate containing types.

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To be continued

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COLLECTING AND IDENTIFYING DIATOMS. - I.

by R. Fraser Bastow, F.R.M.S.

The REV. R. FRASER BASTOW'S papers on diatoms have been published recently by several British Scientific Societies; and in collaboration with DR. FR. HUSTEDT, the greatest diatomist of all time, as well as with the cooperation of the British Museum and the Royal Society, he has good reason to hope that "THE FRESHWATER DIATOM FLORA OF THE BRITISH ISLES" will soon be available for world-wide distribution. Dr. Hustedt has promised that this will be one of his greatest works.

It is probable that everyone, who possesses a microscope and a few slides, has diatoms in his collection, and knows something at least of the skill that is required to show them to the best advantage. In most cases they will be slides that have been bought, and which were probaply made by some professional mounter many years ago, the prices now paid for them being in no way commensurate with their original value, which would have to provide a fitting reward for so much skill and patience. There may yet be one or two enthusists, here and there, who delight in setting up these exquisite creatures in similar array, but let it not be supposed that this is the ideal of every diatom enthusiast.

I am much too impatient, and much too clumsy, ever to become an artistic mounter; my choice is in another direction.

The collection and identification of diatoms is a matter of first rate importance to the study of ecology, which study is an urgent requirement of modern times. Data, respecting the distribution of these ubiquitous organisms, is likely to provide a wealth of information in the field of botanical research. But besides this, it might be difficult to name a science that lends itself so easily to ecological study. The latest phase in the study has to do with environment.

The abode of diatoms is wherever moisture is found: and the presence of species has much to do with its chemical constituents and physical condition. If records, therefore, are intended to be of the utmost value, they should in every case give the chlorine (salt) and acidity values (PH.) of the waters in which they are found. These appear to be the two prime factors of their environment and their values are easy to obtain.

Then there are also distinct genera and species to be found in mosses, others on the roots of hepaticae and ferns, some also on dripping rocks; but their principal habitat is amongst the algae, wherever these are found.

The latest researches have classified the diatoms as having about eighty-five genera; the species, varieties and forms are of course mul-

titudinous, but, carrying the observations along certain well marked lines, the differentiation of the genera is not at all difficult, nor, in general, that of the species and forms.

But it is only fair to say that the identification of some diatoms will almost baffle the ingenuity of the most patient and careful observer. To the advanced student this may be one of their greatest charms.

It has long been the practice to specify diatoms as marine, brackish, freshwater, fossil, sub-fossil, sub-aerial etc., but it is doubtful whether these appellations are altogether justifiable, and whether such particular distinctions can be maintained, except in so far as any have never been known to exist in living form elsewhere.

Marine diatoms are probably more met with in collections, because many of the popular species are easier to mount in picturesque form, and also able to be resolved in moderate fashion with inexpensive objectives. The true resolution of many freshwater species still requires even better objectives than are yet to be acquired, and still defy even the most up-to-date methods of illumination and observation. There is much to be said, however, for grouping the diatoms together into one whole, irrespective of their peculiar habitats.

Localities of fossil diatoms in the British Isles are very few indeed; none have been observed in Devon, where I have been working. This paper therefore has no bearing whatever on that which appeared in Vol. 4., No. 2. of these notes.

It should not be assumed that a characteristic diatom flora is anywhere to be found, though one region will most certainly be found to be richer than another, with regard to the species of certain genera. The local conditions that may be supposed to give rise to abnormal forms are always very interesting. There must be a very mysterious cause for certain species being particularly subject to abnormal growth, whilst it is scarcely ever found in the vast majority of species. It may be that some species are naturally fitted to survive, even with abnormal growth, in an environment where others would quickly perish, but what this particular endowment may be is at present a mystery.

Identifying diatoms necessitates having one or two books of reference. The Diatomaceae of Philadelphia and Vicinity by Boyer, and Bacillariophyta (Diatomeae) by Hustedt are to be particularly recommended. These two works will be found to embrace very many of the marine and freshwater species, and they have copious illustrations, that are generally true to type. The recognition of the genera will soon be mastered; their distinctive features will soon be realized; and though the same may truly be said of the species, these have, however, the quality of running in and out of each other, that is as troublesome as it is interesting to the recorder.

The beginner could not do better than stand on the margin of some pond, pour a tumblerful of the water into the pond from a height of a yard

or so, and take up a tumblerful of the cloudy water that results.

This is almost sure to contain several genera and species of diatoms. In order to rid it of much of the unwanted matter, pour it into another glass through a filter, such as those that are used in cream separating machines. A pad of three or four of these cotton filters will allow most of the diatoms to pass, keeping back much of the mud.

In such condition the microscope would only be able to reveal the outward forms of a few species. In order to identify them, they will have to be cleaned.

When a sufficient quantity of the unwanted matter has been eradicated, the water, in which the diatoms are suspended, must be well acidified with sulphuric acid. Then add a few grains of potassium permanganate and stir well. It will simplify matters if a bottle of dissolved permanganate is kept available. If the pink diatom suspension should clear in the course of a minute or two, add a little more permanganate, and repeat if necessary until the sulution shows no further signs of clearing. Then bleach with a few grains of oxalic acid; add a few drops of strong ammonia; and proceed to separating and washing the diatoms. This is done by allowing the diatoms etc. to settle for a few minutes, pouring off the water, and adding fresh, repeating the process at least half a dozen times.

Great regard must be given to avoiding the possibility of diatoms from one gathering appearing in another, which they are bound to do unless precautions are taken. That is why I use tumblers; they are easily cleaned. But the principal offender is the dipping rod, which must always be avoided. Instead, use an ordinary drinking straw, flattened between finger and thumb at the end that is to be dipped into the suspension. The straws may be cut in two in the interests of economy, and those of cellaphane are best. Of course every one is destroyed immediately after use.

By this means, as much of the suspension as needed may be spread on a cleaned cover glass, and quickly evaporated to dryness. The heat applied for this purpose should never be great, or the liquid will boil, and the diatoms will no longer remain spread, but rather massed together, and of course utterly unrecognisable. Have ready at hand a bottle of mounting fluid.

Now warm a glass slip and place in the middle of it a drop of mounting fluid, immediately putting on the cover-glass, diatoms downward. The mounting fluid will quickly spread and occupy the whole space of the coverglass, and it must then be boiled vigorously for about half a minute, at the end of which, the cover-glass and slip may be clamped together with a very weak wire spring, and allowed to cool.

The mounting fluid will have set hard, and after scraping off any surplus gum, the slide will be ready for examination.

At this stage let me say that I always use Sirax as a mounting fluid,

and well diluted with anhydrous toluine. This fluid is very convenient; it has high refractive index and is supposed to be fairly free from the crystallising habit. The slight pressure that the wire spring exerts whilst the mount is hot, almost completely eradicates the chances of air bubbles in the finished slide.

This is, of course, not the best method of cleaning diatoms; it will not separate them like boiling in acid, but it is certainly the most convenient, and a method that anyone can operate without the slightest objection. It is applicable in all cases of fresh gatherings, whether they are washed from stones by syringing into a suitable receptacle, or shaken from algae, liverwort, or mosses in a suitable sized bottle and a little water. In such cases the cotton-wool filtering pads will be serviceable.

It can be claimed for the modern classification of diatoms that it is a good deal more than phenomenal. That suggested by Hendey (Plankton Diatoms of the Southern Seas, 1937, pp. 202-5) claims to show the systematic arrangement of the genera. This arrangement shows the diatoms as a class of Algae, Bacillariophyceae, comprising one order, Bacillariales, which is divided into ten sub-orders.

The species are all according to certain types that have been figured and described by various authors from time to time; so that when any are referred to, it is necessary to state also the name of the author which will sometimes be enclosed in brackets, thereby signifying that a later and probably more correct description has been made by the author whose name will follow. This proceedure renders the nomenclature somewhat complicated, but it affords a wealth of interest to those who have the necessary literature.

Most diatoms have a semi-obscure organ, the raphe, which not only might serve the organism as the channel of nutrition, but may be also its means of locomotion; for only those that have a raphe have this latter faculty. In most cases also this organ, whether it is present or not, whether on both valves of the organism, or only on one, whether it is straight or curved, simple or convex, whether the cross-markings (striae) relative to it reach it or not, and its general appearance taken as a whole, all these are factors of use in determining to what genus or species a diatom may be said to belong. The raphe is not always centrally placed: In Cymbellae its position is always asymetrical, though sometimes scarcely noticeably so; in Nitzschiae it is in very close proximity to one edge of the organism, forming a sort of a kiel; in Cymatapleura and Surirella it traverses the whole organism in somewhat close proximity to its outer edge, and appears to have appendages travelling inwards. It is worth while making a close study of the raphe, as its nature, form and relative position are of so much importance in diatom identification.

Though, in general, diatoms keep shape to certain definite type forms, they are subject in more or less degree to varying shapes and

proportions; and though the striae are generally of fixed frequency for all respective species and varieties, such frequencies cannot always be reckoned on. In diminishing sizes of frustule the striae are said to become closer together. The frequency is reckoned as so many striae in ten microns; but certain variations are always allowed, as the frequency nearly always varies in different parts, becoming very much closer as the apex of the diatom is being reached.

In closing these few general remarks, I would say how utterly impossible it would be in a paper of this kind to gave any useful advice for the identification of species in particular; but I hope that it will succeed in whetting the appetite of some microscopists for a study of the science, which is not only a pleasurable pursuit, but one also that is full of interest and surprises, so needful and deserving of a greater following.

(To be continued)

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The Use of the Microscope in the Study of Mosses

Cloyd Burnley Stifler

This paper on mosses is not intended to be used for the identification of moss plants but to suggest some of the details in the structure of various parts of the moss plant whose study under the microscope is necessary in order to identify it as to genus and species.

Sphagnum or peat moss and the liverworts, as interesting as the leafy mosses, will not be discussed although the identification of the sphagnums demands very careful use of the microscope and the study of all types of mosses becomes a very interesting and time consuming hobby.

In order to identify any moss, it is necessary to learn many details of its structure. Some of these are macroscopic, and can be seen with the naked eye or with a hand lens. Others are so minute that the use of a compound microscope is essential for their study.

A moss would generally be defined as a small green plant with a leafy stem, growing on moist soil or rocks, the bark of trees or even in water in streams and springs. Their leaves are usually green, but in some mosses growing on rocks, they appear to be nearly black.

Moss stems may be upright and branch at an acute angle or they may be horizontal and branch pinnately, reminding one of a feather.

At certain seasons, mosses produce brownish capsules which may be sessile, or have very short stems or long stems.

In these capsules the spores are developed and the mechanism for their distribution is one of the characteristics of a genus and has been used in the keys for the identification of mosses.

Specimens of moss with capsules are more easily identified than sterile ones.

In the plant world mosses belong to a group known as Bryophytes. They are the simplest plants having stems and leaves.

This group includes the liverworts or hepatics and the sphagnums or peat mosses as well as our leafy mosses.

These bryophytes are placed just above the Thallophytes which means the fungi and algae and just below the pteridophytes which includes the ferns and fern allies.

The bryophytes and pteridophytes have two generations in their life cycle, an asexual one and a sexual one.

Among the bryophytes, the leafy plant is the sexual stage (gametophyte) and bears the sex organs.

The simple little capsule with its bare stem (seta) is the asexual stage (sporophyte) and produces the spores.

Among the pteridophytes, the sexual stage (gametophyte) bearing the sex organs (antheridia and archegonia) is an almost microscopical flat green leaf bearing these organs on its under surface and the asexual stage (the sporophyte) is the green frond by which we recognize ferns.

To mention a few of the structural details which are made use of in identifying a moss, one might begin with the leafy stem. Observe its cross section under the microscope, note the pattern of the attachment of the leaves and their arrangement on the stem.

Note the shape and size of the cells that compose them and, as noted, the way in which they are attached to the stem. Note also the thickness of the cell walls and whether they are smooth or warted.

Observe whether the cells of the leaf are alike or are of different shapes, sizes, and colors, in different parts of the leaf. Note whether the leaf has a midrib (costa). It may have one, two, or none. If it has a costa, is it shorter than the leaf, or as long as it is, or does it extend beyond the tip as a spine? Does it have parallel ridges of green cells above the costa? How many parallel ridges, and how many cells high are they?

Do the leaves have a border of cells of a different shape? Is the edge smooth or toothed? Is it plane involute or revolute?

Is the leaf only one cell thick, or are there several layers of cells? Is it attached to the stem on a straight line, or are the sides decurrent on the stem? Are there any little threads similar to roots (radicles) on the stem?

The leaves at the top, middle, and base of the stem, or the branches, vary and the student should always choose one from the middle of the stem or branch for study.

These are a few of the things we must know, but there are other details to observe.

Does the leafy plant have both kinds of sex organs on it, or are they on separate plants. These organs (antheridia, male) (archegonia, female), grow in the tufts of leaves at the tip of the stem of the upright moss which bears the seta and capsule at its tip (acrocarpous moss) or on a short branch of a horizontal one (pleurocarpous moss).

If there is a capsule present, we know at once that the archegonia was there as the capsule develops in it, but to find these sex organs, if there is

no capsule present, one must separate the terminal leaves and look for them there.

The spermatozoids developing in the antheridia can only be seen under the microscope. They are colorless cells spirally coiled and have two whip-lash cilia at the tip by which, after ejection from the antheridia, they can swim in moisture (dew or rain). They are attracted, probably chemically, to the open tube of the archegonium, down which they swim, but only one spermatozoa fertilizes the ripe egg at its base. This fertilized egg, remaining at the base of the archegonium, develops into the sporophyte (capsule and seta). The archegonium remains attached to the gametophyte, obtaining the greater part of its nourishment from it. The spores develop in the capsule, from which they are scattered.

If a spore falls on moist ground or humus, it germinates, sending out small green threads which branch and finally buds appear on them. These buds develop into the gametophyte or leafy plant, thus completing the life cycle.

Asexual reproduction of moss occurs in other ways - a small piece of a leafy stem will grow into another leafy plant if it has proper soil and moisture just as a slip of geranium grows. Even small bits of crushed parts of dried specimens may grow if given proper growing conditions of moisture, temperature, etc.

Some mosses produce groups of cells (gemmae) in cups of leaves at the tips of stems or in a ball at the tip of a leafless stem, or groups of cells, similar to spores of some fungi, develop on the leaf surface. These also can produce new plants.

Certain details about the sporophyte should be noted. As it develops in the archegonium and elongates, the tube of the archegonium is ruptured and its tip is carried up as a covering at the top of the capsule. It is called the calyptra. Its shape and character vary in the different genera. It may be conical, and if split on one side is termed culcullate, or it may be shaped like a beret and may have a long or short beak at the center. This type is said to be mitrate. The material of which the calyptra is composed may be a smooth membrane or may consist of fine silky fibres, running from the tip to the bottom of the cone, as in the hairy cap mosses, or stiff fibres may project from the calyptra at varying angles.

As the spores ripen, the calyptra falls off, exposing the capsule, whose shape varies from cylindrical to short or long elliptical or even cubical or globose, and its surface is usually smooth. At maturity, in a few genera, it splits open regularly, or with irregular fissures, through which the spores escape.

In most genera, however, the falling of the calyptra exposes a cap (the operculum) at the tip of the capsule, and between this and the rim of

the cup of the capsule, there is in some species a more or less elastic ring (the annulus) which may fall away when the operculum does. The pattern of the cells in the annulus is characteristic. The operculum, like the calyptra, may be beaked or not. When it is removed, there is exposed a more or less open space at the end of the capsule. The opening of the capsule does not have a smooth rim but bears a circle of teeth (the peristome). These teeth vary as to size, shape, and sculpturing. The number of teeth varies in different genera from 4 to 64, but they always occur in multiples of 4. These teeth may be short or long, free at the tips, attached to each other at the tips, or to a membranous diaphragm, or to the membrane at the end of a plug of material in the center of the capsule (the columella).

The spores are developed in the cavity between this columella and the outer wall of the capsule. Their distribution is regulated by the opening and closing of the apertures between the teeth, which are hygroscopic. In dry weather they separate, allowing the spores to be carried away by air currents, but they come together when the air is moist, keeping the spores dry.

The teeth, as noted above, vary as to size, arrangement, color, and sculpturing. Each tooth may stand by itself, they may be in pairs, or they may be split for part of their length. They may form in one circle, or two circles, and may have appendages.

To study the capsule in detail, one must use a microscope. The spores are green or brown and usually globose. Their surfaces may be smooth or rough. Their diameter varies from 10 - 20 microns.

The identification of specimens would be easier if, at the beginning of his study, the student would examine carefully some of the books about them. It is important that he should read the introduction carefully and observe the carefully drawn illustrations, which will show the differences in structure that have been mentioned here.

There are many books about the mosses to be found in libraries and after looking them over, the student can decide which book is best for him.

When specimens are collected, they should be kept separate, and records kept which state the place of collection, date and habitat. This information is necessary for the final labels, together with the name of the collector and identifier. Remember to get specimens with fruit, if possible.

One advantage of studying mosses is that specimens may be dried and kept for study, as they regain their size and color if placed in water, especially hot water.

The illustrations accompanying this article are not drawn to any scale and are merely to suggest what the student may find when studying a moss.

Among the more easily understood books on mosses for a student, I would suggest the following:

I. *Mosses with a Hand Lens* by A. J. Grout.

II. *Mosses with a Hand Lens and Microscope* by A. J. Grout

III. *How to Know the Mosses,* a popular guide to the mosses of the North Eastern United States by Elizabeth Marie Dunham, Published by Houghton Mifflin Company. The introduction is good, also the description of genera and species. Genera are listed by numerals which are used in the keys. These keys are based on habitat as well as leaves and capsules.

IV. *How to Know the Mosses* by Henry S. Conard. This book can be obtained in paper covers with spiral binder and has a black and white picture of the moss in the key. Published by H. E. Jaques, Mt. Pleasant, Iowa.

V. *The Student's Handbook of British Mosses* by H. N. Dixon. Illustrated by H. G. Jamieson. This has an excellent introduction and good illustrations.

VI. *Mosses and Lichens* by Nina L. Marshall. This belongs in The Nature Library, published by Doubleday, Page & Co. It deals only with the commoner mosses.

Plate I, see page 27.

Plate II, see page 28.

PLATE I

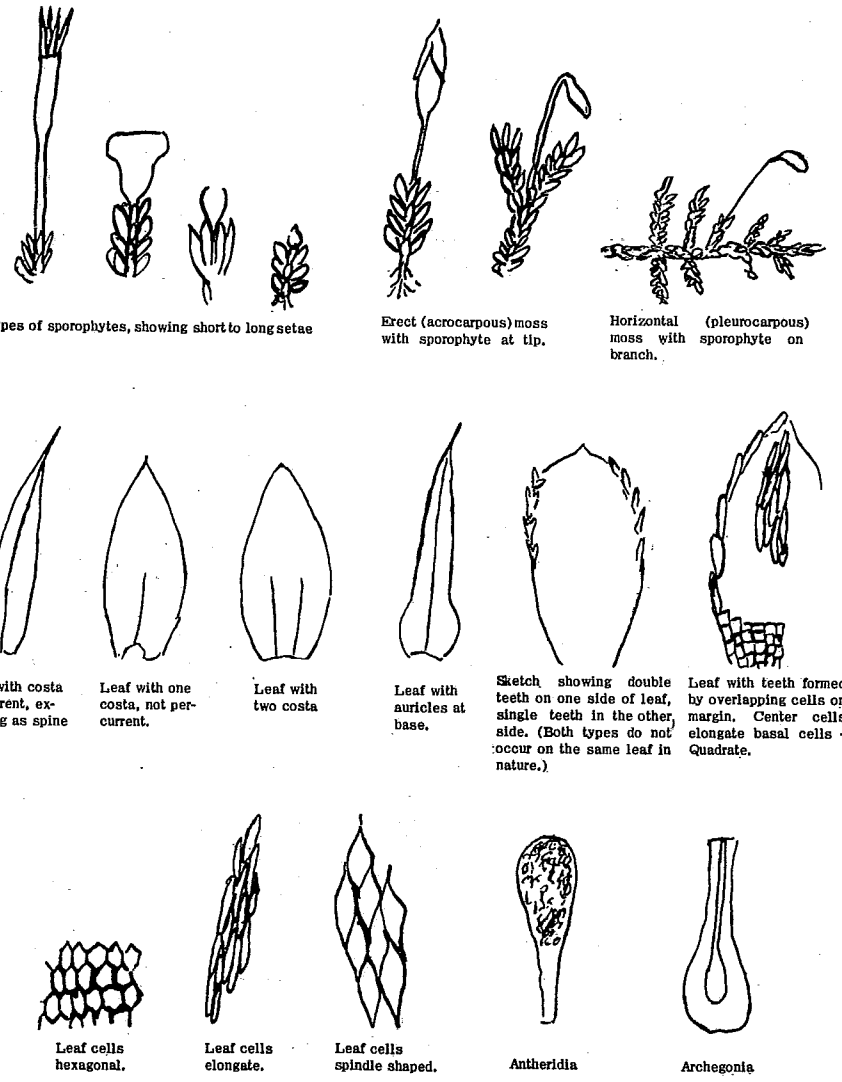
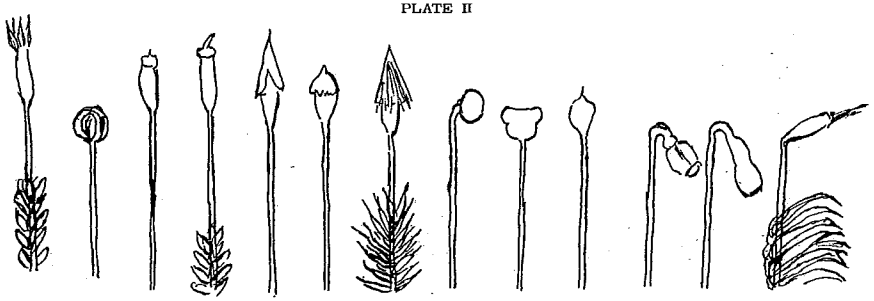
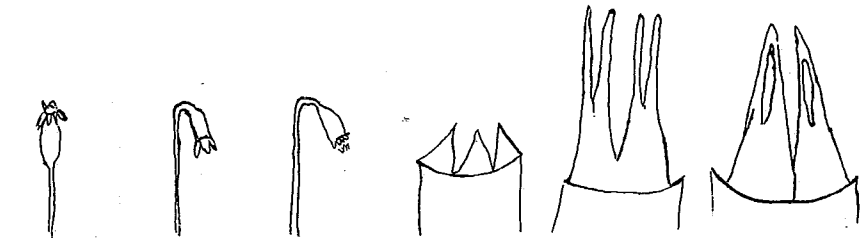


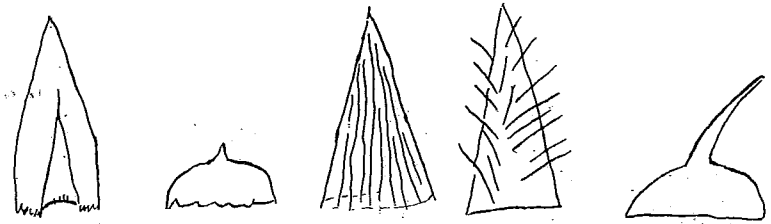
PLATE II



Types of sporophytes



Capsule with teeth revolute | Capsule pendant (1 circle of teeth) | Capsule pendant (2 circles of teeth) | 3 simple peristome teeth | 2 peristome teeth divided ½ way to base | 2 peristome teeth with areolated tip



Calyptra - cuculiate | Calyptra - mitrate with short beak | Calyptra of hairy cap moss | Calyptra of orthotrichum family | Calyptra mitrate - with long beak.

These are not accurate sketches, but suggestions.

ROTIFER CHATS

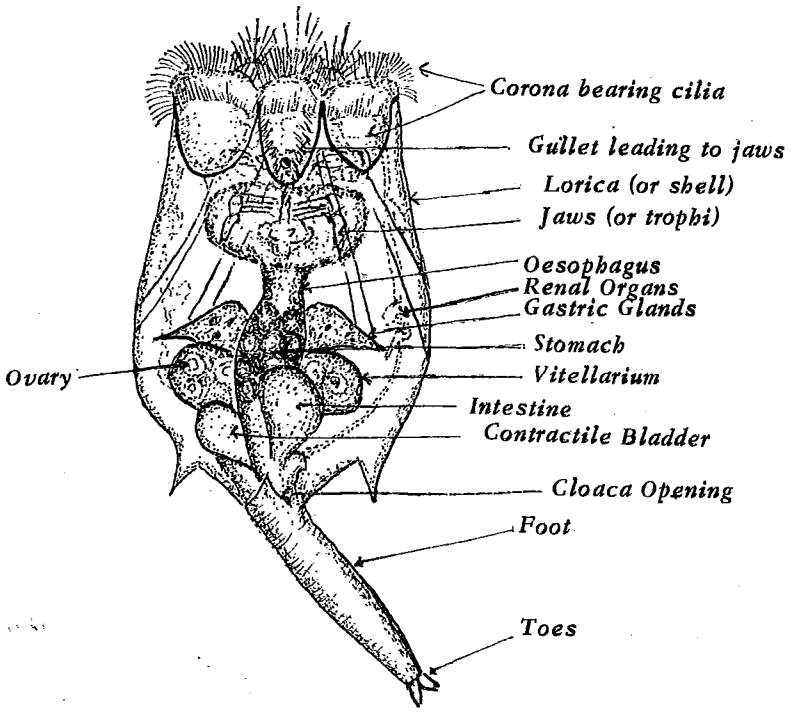
C. Rudlin, F.R.M.S., M.A.M.S.

May I start this series of "chats" by saying that they are not intended for the advanced student of the rotifer, but for those who are just starting this study and are looking around for some simple literature to help them on their way, and also for those who like to take a collecting net and bottle to the pond, just for the sheer joy of beholding under the microscope the "wonders of nature" that are to be found in a tiny drop of water, and who are sufficiently interested to like to be able to identify the rotifers they find therein.

Putting it shortly, then, I hope to try and write the kind of article I should very much have liked to have had available when I first started in this line. I have found that many would-be rotifer students have been put off by the highly scientifically correct articles and lists of species that one sees on this subject, and also by people who, as an old friend of mine once said, "try to blind one with science." I would like to assure anyone who has a little time to spare that they would like to devote to this study, that they can have lots of fun apart from, or in addition to, useful study, without having to attend biological classes (although of course this would be a great help if one had the time and facilities to do so) by using their powers of observation coupled with plenty of patience and the available literature (on which I shall have more to say in a later chat). Also, I shall be only too pleased to help at any time anyone who would care to write to me c/o The Editor of Micro Notes.

Although I intend to write these articles in a plain, not too technical manner, I shall endeavor to make all descriptions, drawings of species, etc., as up-to-date and correct as possible, and if anyone objects to what I say in these chats or thinks I am wrong on any subject, I only hope that he (or she) will write and say so, as by doing this they can be of help to us all.

The Rotifera were first observed in the latter years of the 17th century by the pioneer microscopists, who called them "wheelbearers", since one of the first rotifers discovered was one of the Bdelloids, probably of the genus Rotaria, whose corona consists mainly of two circular discs surrounded by cilia, borne on pedicels or short stems, which resemble two small cogwheels in motion when the animal is swimming or is anchored by its toes and feeding. Since then, owing to their great beauty, interesting habits, and to the ease with which they can be procured, the Rotifera have been great favorites with many amateur micro-biologists the world over. They are common everywhere there is water; in lakes, rivers, ponds, ditches, birdbaths, and often even in roof guttering and mosses.



EXTERNAL AND INTERNAL ANATOMY OF A ROTIFER

POSITION IN THE ANIMAL KINGDOM

The Rotifera have in the past been classed with the Infusoria, and they are also stated by some Zoologists to be related to the worms and Polyzoa. Grove & Newell, in "Animal Biology", put them in a Phylum of their own.

The Rotifera are a peculiar group, probably one of Nature's offshoots, which appear to have come down from prehistoric times in very much the same form as they have today. This I think is rather borne out by the fact that the same species, or many of them, are found all over the world, and there are very few, if any, species which are localized in any particular country. This is particularly noticeable in the island continent of Australia, where other forms of animal life are in many respects much unlike those found in other parts of the world (i.e., marsupials or pouchbearers, etc.) but the known rotifer species are the same as found elsewhere.

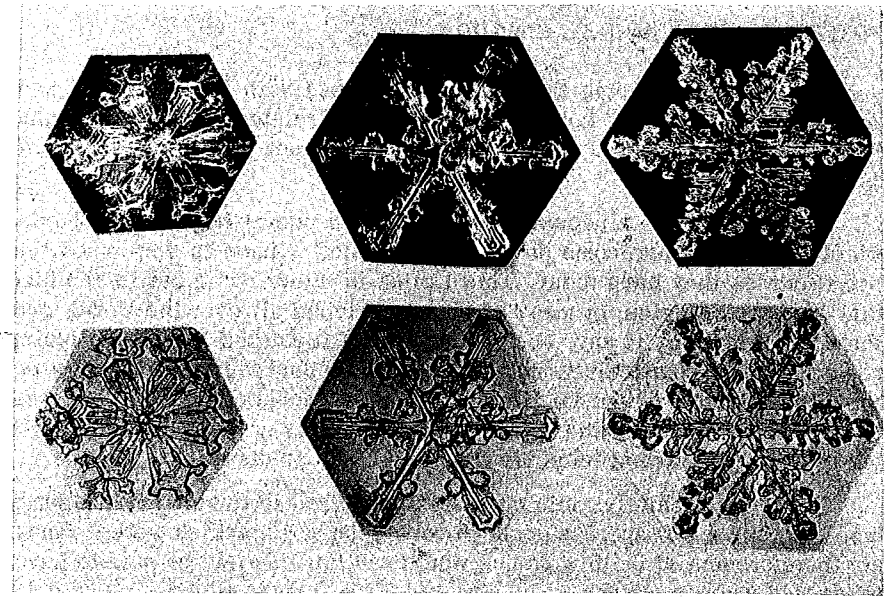
Rotifera are chiefly confined to fresh water; some few are marine, some brackish, and a few species occur in both fresh and brackish waters. They are therefore one of the few groups which can fairly safely be said to have originated in fresh water.

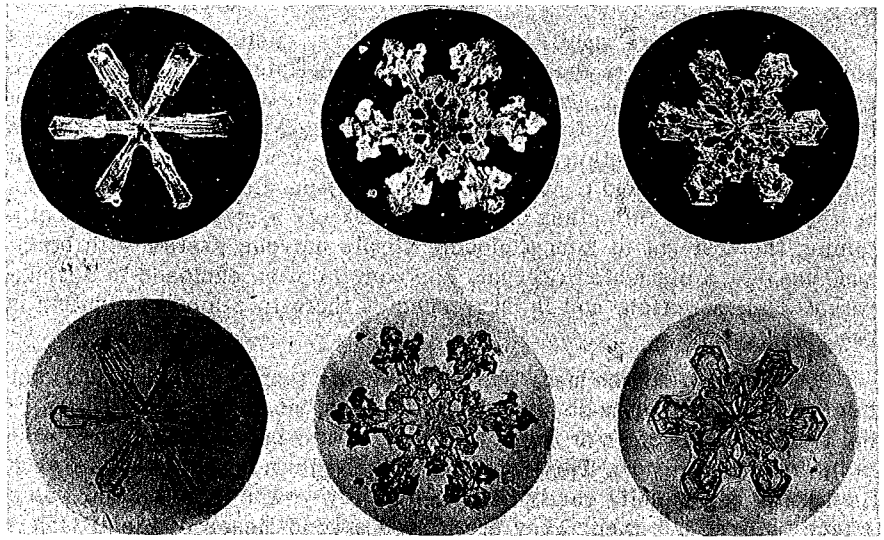
ANATOMY

The Rotifera are small, if not the smallest, Metazoa or many-celled animals (distinct from the Protozoa or non-cellular animals, in which specialized parts of the same cell carry out all the necessary functions of the animal). Briefly, a rotifer is a minute animal, typically with a ciliated trochal disc for locomotion and food collection, a complete alimentary canal with anterior mouth and posterior anus, and a muscular pharynx with jaws or trophi unique to this class of animal; excretory system with flame cells joining the rear gut to form a cloaca; simple nervous system with brain; and, usually, a pigmented eyespot. The body is often enclosed in a transparent shell or lorica, and in many species there is a foot terminating in two toes.

There are of course many species of rotifer (approximately 1,500) but this description fits most species. One of the most striking features of the rotifera is the jaws or trophi, which is not to be found anywhere else in the Animal Kingdom. The movement of these jaws or trophi can be seen (in many species) quite easily even under a low-power objective such as a 1 inch or even a 2 inch. They are indeed a good means of identifying a rotifer (excepting the male, which in most species has no jaws) from any other small animal. In addition rotifera, unlike the protozoa, have the cilia used for locomotion on the front part of the body or head only; this also is helpful in making reasonably sure whether the animal at which you are looking is, or is not, a rotifer.

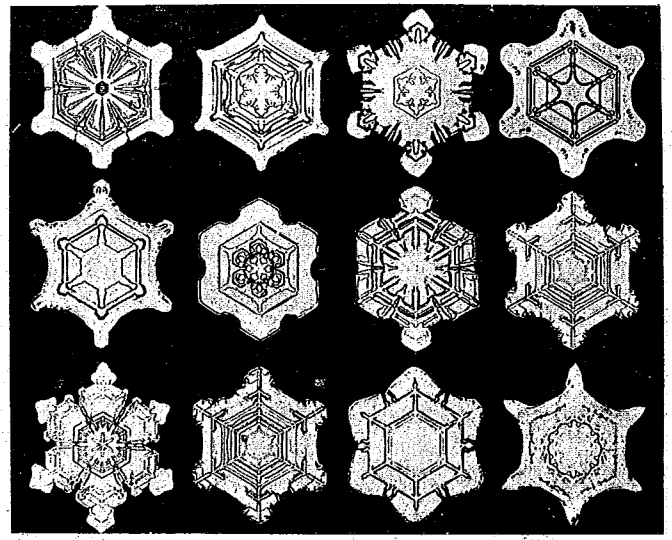
(to be continued)





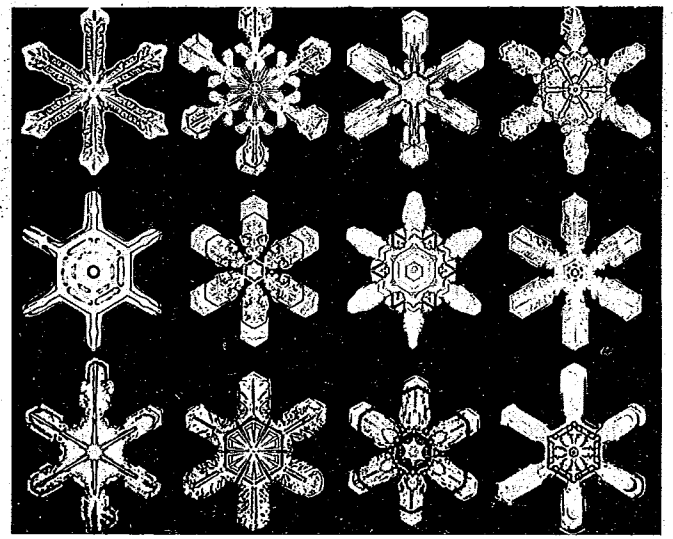
581344 6 SNOWFLAKE CASTS MADE BY VINCENT J. SCHAEFER OF G-E RESEARCH LABORATORY. VIEW (ENLARGED 20 DIAMETERS) WITH BOTH REFLECTED AND TRANSMITTED LIGHT UNDER MICROSCOPE.

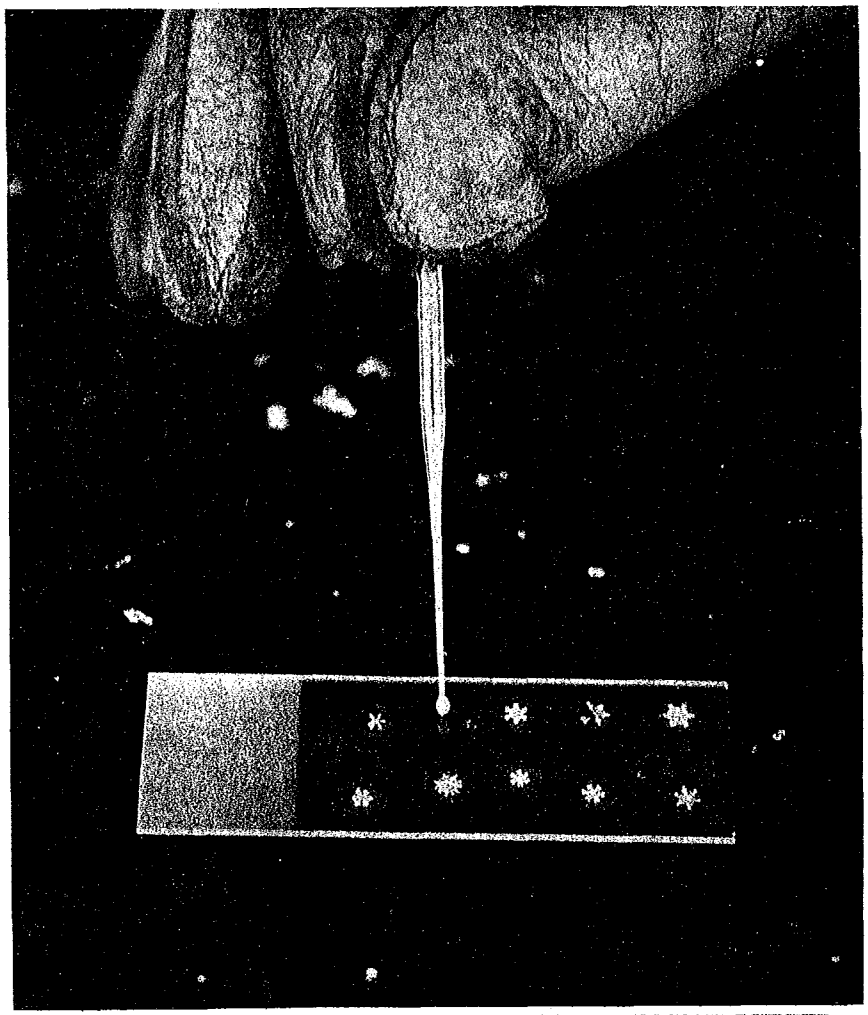
FILING NO.8851 551.57 3-10-41



Illustrations from Bentley and Humphreys book, "Snow Crystals":

This book, "Snow Crystals", by W. A. Bentley and W. J. Humphreys (McGraw-Hill, 1931), contains photomicrographs of thousands of beautiful snow crystal specimens, made over a period of 50 years by W. A. Bentley of Jericho, Vermont. No two of the designs are alike, and often show a remarkable mathematical regularity in their forms. (Plate reproduced courtesy of the publishers and the United States Weather Bureau.)





(From General News Bureau, GENERAL ELECTRIC COMPANY, Schenectady 5, N. Y.)

Plastic replicas of snowflakes being prepared on glass slide with technique developed by Dr. V. J. Schaefer, General Electric scientist. After snowflake is caught on slide, a single drop of plastic solution of Formvar is placed on snowflake. Solution dries, leaving hard plastic cast of perfect reproduction of snowflake.

MAKING PLASTIC REPLICAS OF SNOW CRYSTALS

By Ben F. Laposky of Cherokee, Iowa.

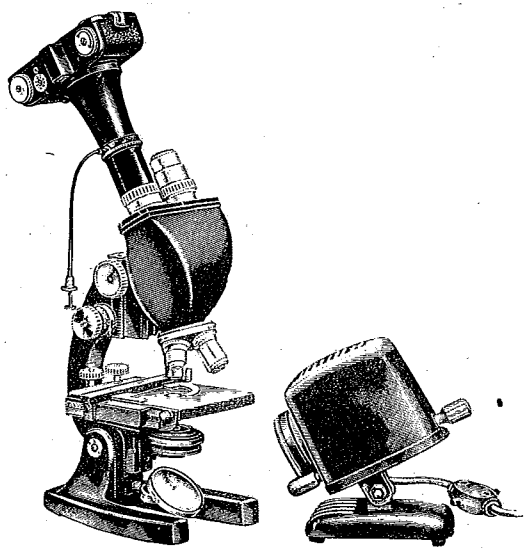
The study of snow crystals under a microscope or making photomicrographs of their beautiful designs is a cold and painstaking task. However, a method developed some years ago by Dr. Vincent Schaefer of the General Electric Laboratory at Schenectedy greatly aids the microscopist in this endeavor. It is to make plastic casts of snow crystals on microscope slides. These slides may then be photographed more easily or even used for microprojection, a feat which would be impossible with real snow crystals.

Schaefer's method is to use a 1% solution of formvar 15-95, a polyvinyl formal resin, dissolved in ethylene dichloride. This solution must be chilled below freezing before using. The crystals are caught on a board covered with black cloth, and then a glass rod or wire dipped in the plastic solution is touched to likely looking specimens which are then transferred to a microscope slide. Another drop of solution is placed on the crystal on the slide, as shown in the photograph of Dr. Schaefer making a slide. The solvent evaporates quickly, leaving the flake encased in a shell of plastic resin. Later the water forming the snow crystal evaporates thru the plastic's pores. The thickness of the shell of the replica is estimated at around 20,000 angstrom units by Dr. Schaefer.

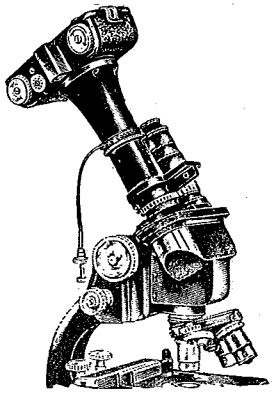
All of this work must be done, of course, out of doors or in a building at the same temperature as the outdoor air, and all materials used kept very cold. We have found in this section of the country (Iowa) that the best crystals fall during snowstorms when the temperature ranges between 10 degrees and 20 degrees F., and generally when the wind is northeast. Above 20 degrees the crystals melt more easily and do not always show the fine detail of the colder ones---they also tend to be more of the open or branched forms.

A couple of little gadgets we have found helpful in making snow crystal slides are a spacing guide and a scraper for removing unwanted specimens. The guide is of black cardboard marked in ¼" squares in white ink, the crystal slide being placed on it while making the replicas. A small scraper, about 3/16" wide, can be made of a bit of razor blade mounted on a matchstick---this will easily remove damaged or uninteresting replicas from your slides.

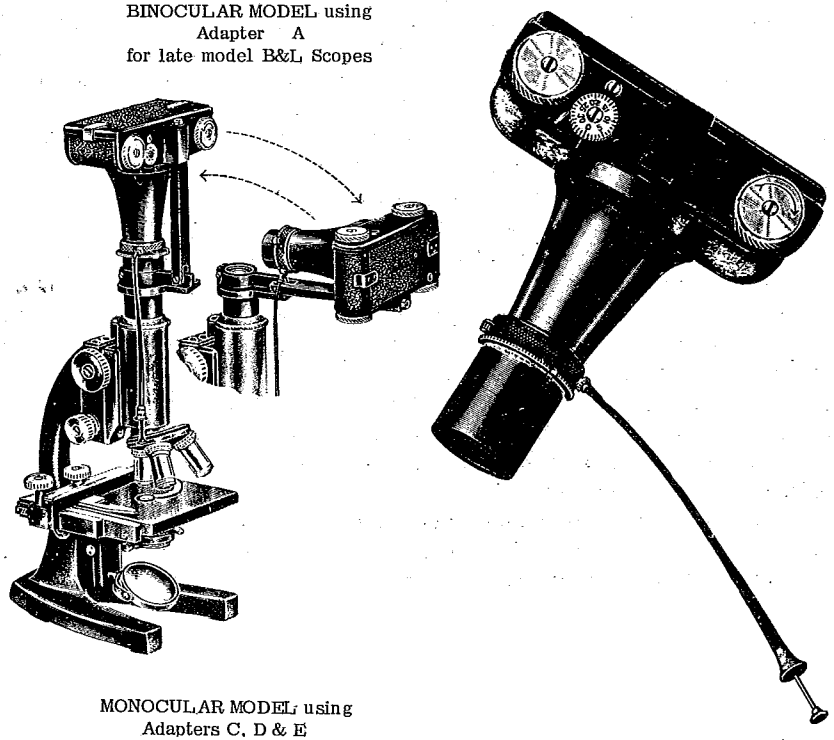
The plastic replica method described above was used by Dr. Schaefer in connection with research on precipitation static for the air force and also in his famous rainmaking experiments.



BINOCULAR MODEL using
Adapter A
for late model B&L Scopes



BINOCULAR MODEL using
Adapter B & D



MONOCULAR MODEL using
Adapters C, D & E

NEW PRODUCTS

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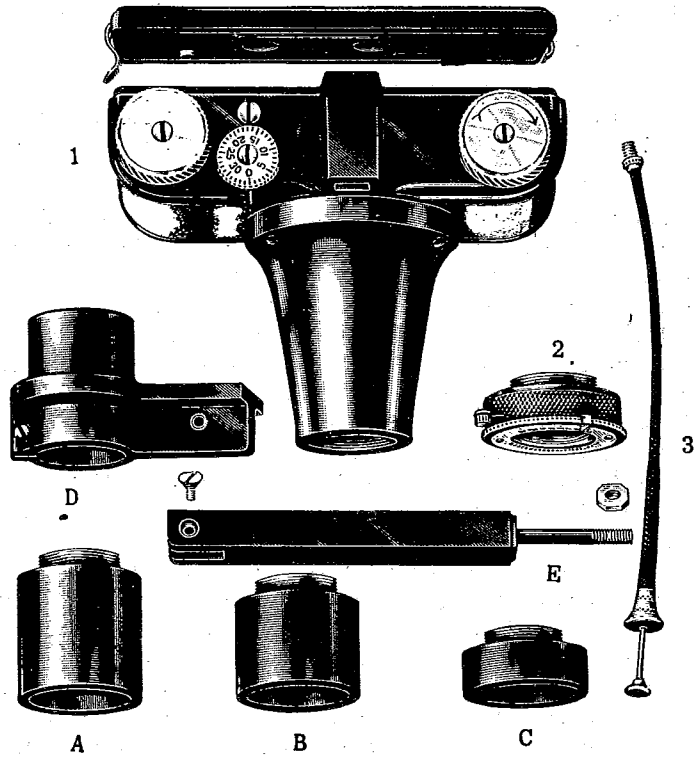
HISTOSLIDE CAMERA FOR PHOTO-MICROGRAPHY

On a recent visit to a Chicago hospital we had an opportunity to observe the combined use of microscopy and photography in routine laboratory operation. The simplicity of the process is worth recording.

A number of microscopes were in use, each with its HISTOSLIDE CAMERA ATTACHMENT. The laboratory technician or the physician simply found what was wanted on the slide, swung the camera into position, snapped the photograph, removed the camera, and was ready for new fields of action. The taking of the photograph consumed as a rule much less time than it took to decide on what to photograph.



The camera is manufactured by HISTOSLIDE CO., INC., 542 Grant Place, Chicago 14, Illinois. The illustrations above and on the opposite page are taken from an attractive booklet obtained from the company who informed us that the price for the camera, complete with adaptors to fit any microscope, is $37.50 with tax included.

BOOK REVIEW

Bouwers, A. 1946 Achievements in Optics pp. 135, being Vol. 1 in Monographs on the Progress of Research in Holland during the War, R. Houwink and J. A. A. Ketelaar, editors. Amsterdam, Elsevier.

This compact volume, the first in a remarkable series intended "as a token of the undaunted spirit of the Netherlands" was begun under the nose of the invader. Its interest for the practicing microscopist is at least twofold, for its consideration of some fundamental problems of geometrical and physical optics which may have long-range effects on optical design and for the introduction of new instruments. Of the latter, reprints in English of Bouwers *Nederlandsche Natuurkundige Vereeniging* paper of October 1943 on his new mirror microscopes and of Zernike's now classical paper "Phase contrast, a new method for the for the microscopic observation of transparent objects" (*Physica, 9:* 686, 1942), place this little book on the must list for science libraries and for senior students of microscopy.

Houwink, A. L., J. B. LePoole and W. A. LeRutte (editorial committee) 1950 Proceedings of the Conference on Electron Microscopy Delft 4-8 July 1949, pp. 188, Delft, Hoogland.

It is fitting that Delft, the city hospitable to Leeuwenhoek's pioneering observations with the light microscope, should be three centuries later the scene of a seminal conference on electron microscopy, drawing a group of more than 200 investigators (from most parts of the non-Sovietized world) presenting 46 papers. The studies range from discussions of present instruments, methods and accomplishments through considerations of the nature of suitable electron sources, lens systems and their aberrations, and resolving power to presagement of developments of coming years. Particularly intriguing are attempts to realize a phase contrast electron microscope (Agar, Revell and Scott of Manchester) and to develop electron diffraction microscopy to realize a resolving power beyond 10 Å (D. Gabor of London).

The editors apologize for the stringent cutting of papers and for the quality of reproduction of photographs in the economy of publication. Whatever has been lost in the cutting, the remainder constitutes a revealing, valuable review of the current state of electron microscopy --- certainly a highly needed supplement to G. H. Scott's section on the preparation of tissues for electron microscopy in the new edition of McClung's *Handbook of Microscopical Technique.* If the editors' disparagement of the reproduction of figures is at all warranted, the originals must in many cases be magnificent.

J. L. Mohr

Department of Zoology
University of Southern California

NEWS FROM THE FIELD

INDUSTRIAL MICROBIOLOGISTS FORM NATIONAL SOCIETY

NEW YORK - Swarming microorganisms have a new scientific society to study them. It is the Society of Industrial Microbiologists, formed during the recent science meeting here.

The new group will pay special attention to microscopic life that destroys clothing, building materials and other substances. The organisms that produce the antibiotics, such as penicillin, yeasts that yield alcohol in beer and liquor, and bacteria that produce useful chemicals are in the field of the society.

Dr. Charles Thom of Jeffersonville, N.Y., is the first president.

- Science Service

NEW SUITS FOR NEWTS WITH HIGH FREQUENCY SOUND WAVES

STORRS, Conn., - There is a new way to skin a newt. Very high frequency sound waves will make the little amphibian shed skin like an onion.

When left to its own schedule, a newt will molt about three times every two months. But when the little creature is placed in a bottle, and subjected to ultrasonic vibrations for eight to 120 seconds, its shedding rate jumps to a maximum of 7.6 newt-suits per 30 days.

Unlike most of the known ways for skinning cats, the sound wave treatment is not fatal to newts if the energy level is kept within limits, Dr. Hugh Clark of the University of Connecticut has found.

Above 35 watts of energy the dose is fatal, but below this level newts can be sound-vibrated indefinitely without any other observed effect except a rapid sequence of new coats.

Some other conclusions noted by Dr. Clark are:

1. One ultrasonic jolt of 30 watts for one to two minutes keeps a newt on the rapid molt routine for at least 70 days.

2. The vibrations seem to act on the epidermis but no skin effects were noticed.

3. Although treatment seems to stimulate the thyroid gland directly, the pituitary gland shows no activity.

-Science Service

RADIO WAVES USED TO MAKE CHEESE FREE FROM BACTERIA

ITHACA, N.Y., - Cheese can now be made bacteria-free more easily.

This is done by pasteurizing the cheese with radio waves after the cheese has aged, three Cornell scientists have found. The presently used method is to pasteurize the milk from which the cheese is obtained.

It is much easier to rid 10 pounds of cheese of bacteria than to pasteurize the 100 pounds of milk from which it is made. The new method also makes possible the pasteurization of cheese after it has been wrapped, thus giving the consumer an uncontaminated product.

Since the elusive cheddar flavor has only come from aging cheese

made from raw milk, the researchers had hoped to pasteurize old cheese after the raw milk flavor had developed. But the radio frequencies pasteurized only the very young raw milk cheese.

Cheese from the experiments was flavor-tested by competent cheese tasters. Although scores ranged widely, some of them were high, though not equal to an aged cheese made from raw milk.

In their system, Drs. F. V. Kosikowsky, B. L. Herrington, and A. C. Dahlberg placed the cheese between two plates or electrodes carrying a high frequency current. Friction is set up between the cheese molecules by alternating current, raising the temperature to 132 degrees Fahrenheit in a minute or two. Then the cheese is cooled by air. This pasteurizes the cheese, yet leaves enough enzymes and bacteria to develop flavor.

-Science Service

METAL FILMS HELP SEE BIG MOLECULES IN ELECTRON MICROSCOPE

KINGSPORT, Tenn., - Better understanding of large molecules, such as those in rubber, is promised from a technique developed here.

Use of an alloy of aluminum and beryllium when preparing samples to be studied in the electron microscope is said to do the trick. The method, particularly suitable for large particles, was developed by Wilbur Kaye of the Tennessee Eastman Corporation here.

The alloy is used as the mounting surface for the sample that is being examined. Aluminum-beryllium is superior to the collodion or other materials commonly used for support of the specimen, it is claimed. This is because by "alloying these two light metals it is possible to reduce greatly the granularity of structure," Mr. Kaye states. He says that the alloy has advantages because of its high strength, good electrical conductivity, insolubility in nearly all solvents and low density.

-Science Service

HORSESHOE CRAB HAS DELICATE COMPASS IN EYE

NEW HAVEN, Conn., - The lowly, spiny-tailed horseshoe crab has a delicate compass in his bulbous eyes. It is affected by polarized light.

This discovery was reported today by a Yale University zoologist, Dr. Talbot H. Waterman. It could give scientists a clue to the ability of high-flying insects to "see" their way to distant points by invisible polarization of light.

Dr. Waterman found that the compound eye of the horseshoe crab, which is similar to that of many insects, is affected by even a slight change in light polarization.

The zoologist hooked a loudspeaker to nerves leading from the crab's eye, among other instruments used in the intricate experiments. Then he listened to electrical impulses produced when a pinpoint of polarized light was shone into the eye.

How the crab uses his light compass is still not known, Dr. Waterman says in the forthcoming issue of the journal, Science.

-Science Service

GERMANIUM, CHEMICAL FOR INFRARED LENSES, NOW MADE IN PURER FORM

OAK RIDGE, Tenn., - A technique of purifying germanium, the chemical from which lenses for spectacularly improved infrared equipment can be made, was announced here.

Lenses made from germanium transmit invisible heat radiation. The germanium lenses will do this even though they are an inch thick and do not allow ordinary light to pass.

Previous to the discovery of the infrared transmitting qualities of germanium, materials that are attacked by moisture have been used for optical work in the infrared region. During the last war important military applications were found for instruments using infrared radiation.

Dr. R. N. Hall of General Electric Research Laboratory found that germanium could be cooled with the direction of cooling controlled so that most of the impurities were concentrated at either end. Successive recrystallizatoins of the central sections remove impurities to the point where they are almost non-existant.

The extent of their removal is measured by the electrical conductivity, Dr. Hall told the American Physical Society meeting here.

BARNYARD ANIMALS BETTER FED THAN PEOPLE CHEMIST FINDS

WASHINGTON, - Recent developments in feeds for America's farm animals have brought better diets to the barnyard than most people enjoy, the National Farm Chemurgic Council was told here today.

Progress in vitamin studies, particularly on vitamin B-12 in the so-called "animal protein factor," in wider use of amino acids and in knowledge of mineral requirements of farm animals was described by Dr. H. J. Prebluda, nutritional chemist for New York's U. S. Industrial Chemicals Corp. He predicted that the coming decade would be called "the fortified fifties."

"If as much interest could be aroused in feeding our population as in baby chicks and hogs," said Dr. Prebluda, "we would not only be the best fed nation on earth, but we wouldn't worry over crop surpluses."

Dr. Karl D. Butler, farm counselor from Ithaca, N.Y., said much the same thing in a second speech: "Livestock are fed better, from the standpoint of nutrition, than are people."

Science has boosted U.S. food output even though total acreage of vital crops has dropped in the past two decades, he said. Among developments to come, he predicted much greater use of yeast fermentation methods for producing protein foods from present crop and forest wastes.

-Science Service

CHEMICAL SEEN AS HEREDITY CARRIER

NEW YORK, - Heredity is a matter of chemistry, it appears from a discovery by Prof. Arthur W. Pollister of Columbia University. here.

It is a chemical substance within the single cell which probably acts as the carrier of the hereditary units known as genes, Prof. Pollister finds.

This chemical is desoxypentose nucleic acid, or DNA for short. Using a complex machine for photometric chemical analysis, Prof. Pollister was able to determine the relative concentrations of DNA and other substances in the nucleus of a single cell. Within this structure of a few ten-thousandths of an inch, it can be shown that the amount of DNA is less than one-trillionth of an ounce.

The genes are located in the chromosomes of the cell nucleus. Nearly every cell of the body contains at least a double set of chromosomes and a double set of genes. This double set is present because at the fertilization of the egg, two sets of chromosomes and genes were brought together, one from each parent.

"By direct photometric analyses here at the University's laboratories," Dr. Pollister declared, "it has been shown that DNA alone is strictly parallel in amount with the number of sets of chromosomes and genes."

"When analyzed, the very common double-chromosome nuclei of such cells as blood, liver, brain, kidney and glands, have all proved to have the same amount of DNA."

The single-chromosome nuclei of the sex cells, as expected, were found to have just one-half as much DNA. And, as often happens in science as well as other fields, the exceptions eventually helped to prove the rule: the "giant" four chromosome nuclei, and the still rarer "super-giant" eight-chromosome nuclei turned out to have four and eight times as much DNA as the single-chromosome sex cells.

The experiments served to clear up an international disagreement over DNA, Dr. Pollister stated. Previously, French biochemists, by comparing their analyses of DNA in masses of nuclei with the number of billions of nuclei estimated to be in the mass, found indications that the amount of DNA per nucleus might be constant, and about double that of the male sex cells. An American laboratory got results which did not agree with the French claims.

The direct measurements made on single cells in the Columbia laboratories clear up the dispute, Dr. Pollister asserted. They prove conclusively that the French tests are correct, and at the same time offer, in the analysis of the "giant" nuclei, an explanation of the disagreement that existed, he said.

The project also revealed that cells may grow to eight times their usual size without any increase in the amount of DNA; that cancer cells contain the same amount of DNA as normal cells; and that human blood-forming cells always contain exactly the same kind and amount of DNA whether the cells are from

infants, youths, or adults, from persons with extreme anemia, or from those who are recovering from anemia as the result of treatment with Vitamin B-12.

-Science Service

FUTURE SCIENTISTS HAVE VARIED FAMILY BACKGROUNDS

Forty 15- to 18-year old high school seniors will gather here in the early days of March for the Institute of the Ninth Annual Science Talent Search, conducted by Science Clubs of America. Chosen for their potentialities as future research scientists, they will compete for $11,000 in Westinghouse Science Scholarships.

Among the participating finalists will be a 16-year old boy who discovered three unrecorded fish in New York waters, a 18-year old high school athlete who is now completing a three-dimensional table of the chemical elements and a 16-year old girl who has devised a relatively inexpensive chemical treatment for desert soils.

Economic status and occupation of the father seem to have very little to do with the making of a scientist. The fathers of the 40 winners are about evenly divided in professional and non-professional occupations. Mothers of the winners are in general occupied with their duties as homemaker but about 10 find time for full or part-time jobs in addition.

Approximately 57% of the winners' fathers and 35% of their mothers attended college.

Complete details of the annual Science Talent Search may be obtained by writing Science Clubs of America, 1719 N. St., N.W., Washington 6, D. C.

-Science Service

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I SAW "IT" IN MICRO NOTES.

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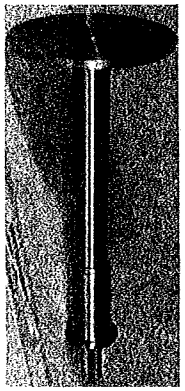
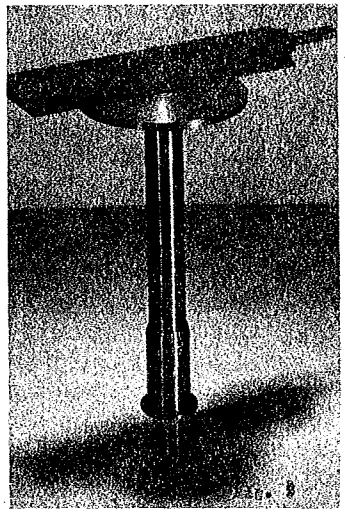
HAND MICROTOME: WELL PATTERN



When only a few thin sections are required this hand microtome, invented a long time ago by Ranvier, is extremely convenient. The tissue is embedded in paraffin, carrot, potato or pith and then placed in the well of the microtome. This is best done by cutting the supporting material into a core which will fit snugly into the well, then slicing it lengthwise and placing the tissue in this cut. If paraffin is used, a paper form is made which has the dimensions of the well, place the tissue in this form, and pour melted but cool paraffin into the form. When hard, the paraffin will support the tissue for cutting. While designed primarily for cutting section of stems and roots, this hand microtome can be used for both animal and vegatable tissue. The feed is accurate and, by means of a micrometer screw, entirely enclosed. Each graduation has a value of about 5 microns. Tissue in size to ½" diameter and 2" long can be handled. After placing the tissue in the well - it is gradually raised by means of the milled head, a section may now be cut. This is repeated until a sufficient number of section are secured.

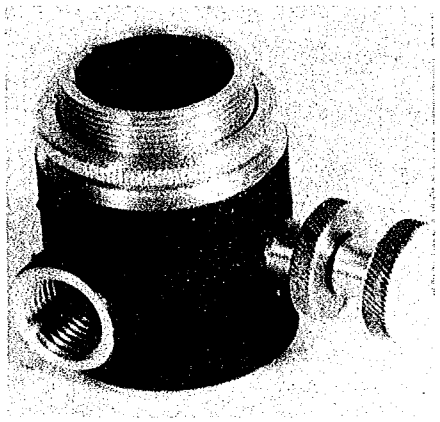


A very, very sharp razor or microtome knife is required. Adjust the thickness of sections cut so that they do not curl. Books on section cutting will give hints of how to get good sections and how these are stained.

Figure 1 shows a Hand Microtome, overall length about 7", which sells for about $6.00.

Figure 2 shows how the knife is held to cut a section with this instrument.

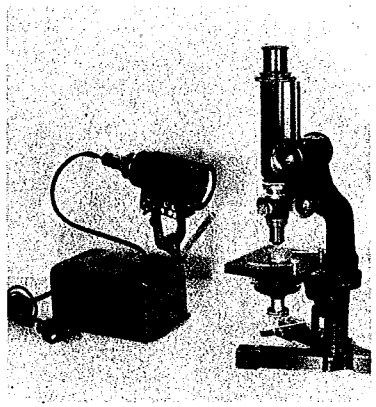
VERTICAL ILLUMINATOR



A simplified vertical illuminator for opaque objects such as polished metal surfaces is a very handy instrument for the microscopist to have around. With it he can see the beautiful crystaline surfaces of etched polished metals. Study of these surfaces is both instructive and interesting. A knowledge of metallography may even be of financial gain to him.

This unit is designed so that it can be screwed into the nosepiece of a standard biological microscope in place of the objective. The objective is then screwed into the lower end of the illuminator which has the standard R.M.S. screw thread.

A collimated beam of bright light is projected upon the coverglass reflector inside the body of the illuminator. This cover is rotated by means of the knob on the unit so that the light is thrown down thru the objective upon the objective. The polished surface of the object reflect light upward into the objective and an image formed of this surface in the eyepiece.



With this type of illuminator the full resolving power of the objective is employed as the cone of light from the back lens is not obstructed by mirror or prism. The plain glass reflector is mounted so that it can be readily replaced or adjusted by means of knob on the side. A lock-nut sets the reflector in place when the correct angle is found. The unit pictured in figure 1 is made of duraluminum and sells for about $7.50.

The general arrangement of the microscope and lame is shown in figure 2.

DISSECTING SETS and MAGNIFIERS

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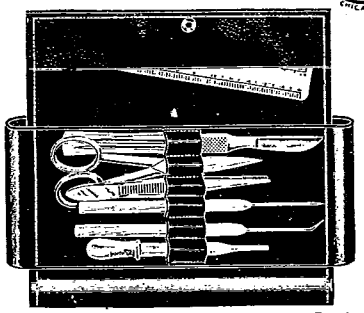
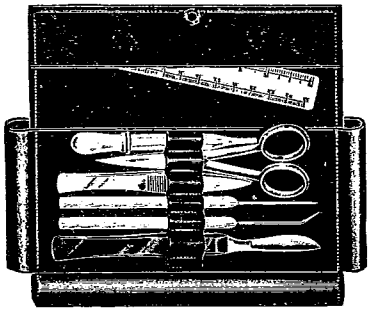


No. 2A—DISSECTING SET .....Ea. $1.90



No. 3B—DISSECTING SET .....Ea. $1.40

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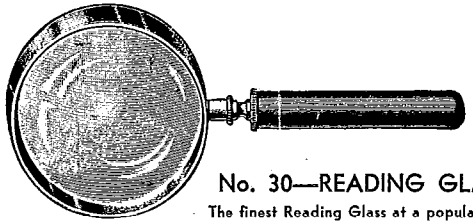

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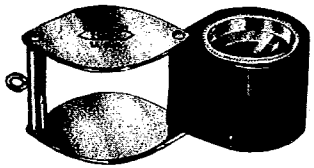
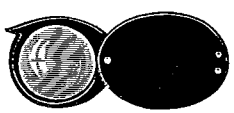
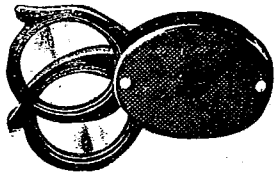
No. 30—4"	"	"	.....Ea. 2.75
No. 30—4½"	"	"	.....Ea. 4.00



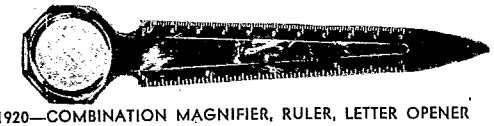
No. 19F—DOUBLET MAGNIFIER, 10X, folding in metal mount. Very popular in botany, geology, engineering, etc.
Each $3.25



No. 19A MAGNIFIER
Folding 5X magnifier, 25 mm. diameter lens in black vulcanite mounting.
Each. $0.50



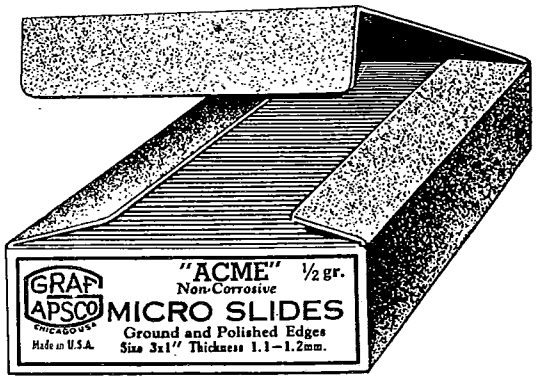
No. 19B FOLDING MAGNIFIER. Like No. 19A but two 25 mm. lenses, 5X and 10X magnifications .....Ea. $0.75



No. 1920—COMBINATION MAGNIFIER, RULER, LETTER OPENER
Good quality lens, 1¼" diameter mounted in attractive plastic handle, magnification 4X, individually boxed. .....Ea. $0.55

BIOLOGY SUPPLIES

MICRO-SLIDES



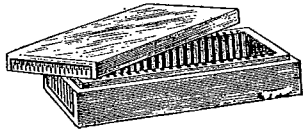
No. A1450
"ACME" MICRO SLIDES
A high quality slide made of American glass, ground edges, uniform thickness, 1.1-1.2 mm., packed ½ gross to box and cellophane sealed to protect against moisture and corrosion.
No. A1450—Slides gross $1.75 net

CLINICAL GRADE SLIDES
Also available in limited quantities are SECONDS of our No. A1450 slides. These are imperfect but useable and recommended for use on smears, etc.
No. 23—Clinical Slides, gr. $1.10 net

CULTURE SLIDES

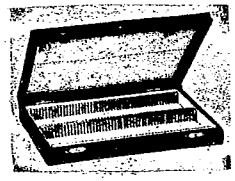
No. A1471 No. A1475 No. A1476

No. A1471—1.5 mm. thick, 1 concavity...doz. $0.90
No. A1472—1.5 mm. thick, 2 concavities.doz. 1.50
No. A1473—3 mm. thick, 1 deep cor cavity. .Ea. $0.20
No. A1475—3 mm. thick, 2 deep concavities .Ea. 0.45
No. A1476—DEEP WELL SLIDE, 1 straight wall concavity..... .....Ea. $0.60



No. 43—SLIDE BOX, all wood with overlapping cover. Capacity, 25, 3"x1" slides.
Each $0.25

COVER GLASSES



No. 45—SLIDE BOX, capacity 100, 3"x1" slides. Wood frame and cardboard backing. Overall size 11¼"x7½".
Each .....$ 1.25

No. 1 Thickness

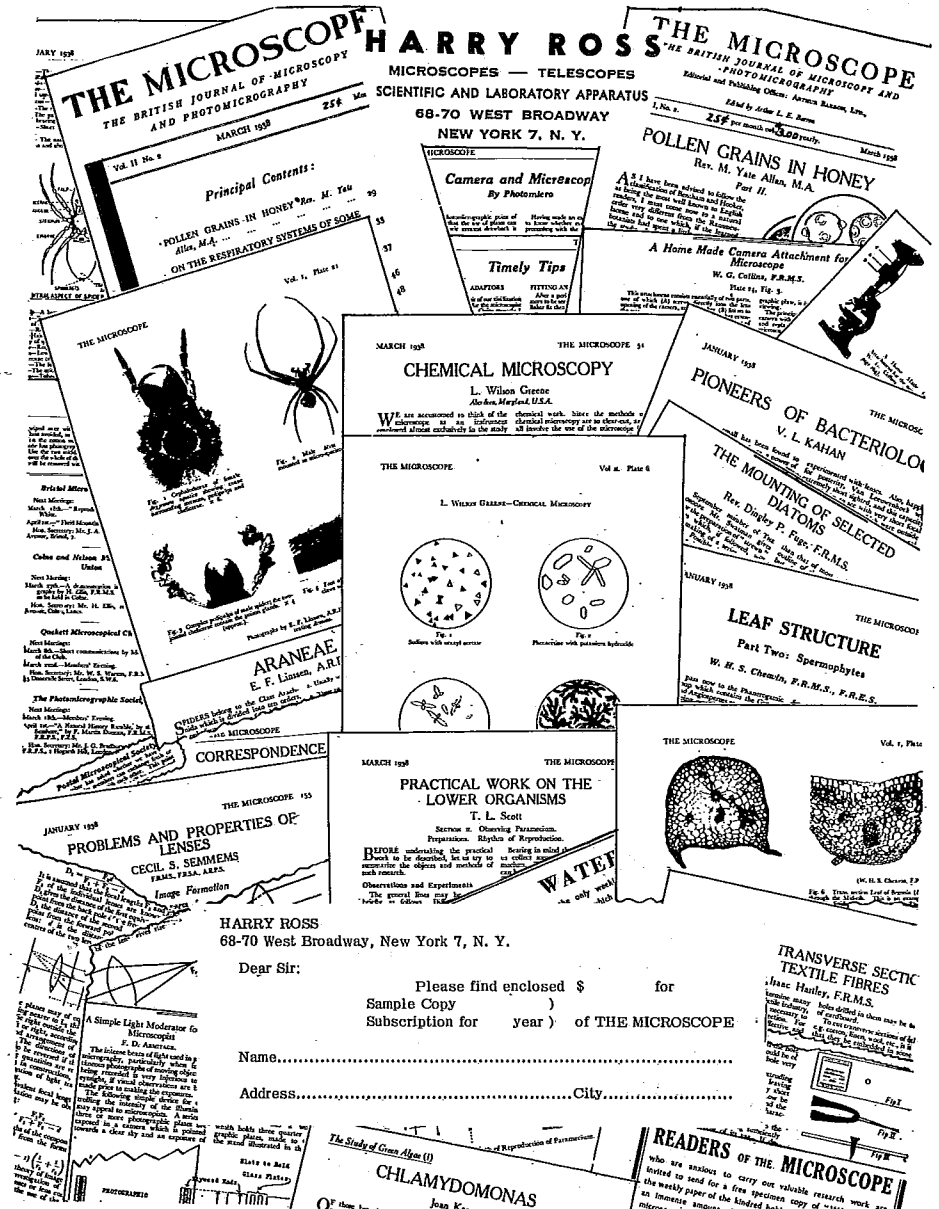
SQUARES	18 mm. 22 mm. 25 mm.	CIRCLES
$2.25 oz.		$2.90 oz.

22 mm x 30, 35, 40, 45, 50 mm rectangular.$2.25 per oz.
24 mm x 30, 35, 50 mm rectangular..... $2.25 per oz.

No. 2 Thickness

SQUARES	18 mm. 22 mm. 25 mm.	CIRCLES
$1.60 oz.		$2.00 oz.

22 mm x 30, 35, 40, 45 or 50 mm rectangular$1.60 per oz.
24 mm x 30, 35, 40, 45 or 50 mm rectangular$1.60 per oz.
10% discount on 50 or more oz.



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