

About a hundred spherical atmometers with mountings like the one here described were in operation, in the Ohio survey mentioned above, during a nineteen-week period in 1929. With the exception of two, the valves of which were accidentally broken, all the instruments operated perfectly throughout the period without the attention of persons specially trained in caring for them.

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THE USE OF N-BUTYL ALCOHOL IN DEHYDRATING WOODY TISSUE FOR PARAFFIN EMBEDDING

THE common procedure in dehydrating, clearing and embedding tissue for cytological examination is, first, to replace the water normally present in the specimen with ethyl alcohol. The alcohol is then replaced by some volatile fluid soluble both in it and in paraffin. In the preparation of plant material for sectioning this fluid is nearly always xylene, which is in turn replaced by melted paraffin. On solidification the paraffin holds and supports the tissue so that it can be sectioned properly.

There are certain limitations to the above technique which make it unsuited for animal cells and for any plant material which contains lignified elements. The higher concentrations of alcohol harden any specimen left in them too long, often before it is completely dehydrated. All the water must be extracted from the specimen before the xylene will penetrate, and this involves the use of absolute alcohol. The xylene itself causes animal cells to shrink and become brittle and so hardens the wood elements that they can not be cut but break and chip the edge of the microtome knife. These disadvantages have been overcome in the preparation of small zoological specimens by using clove oil, cedar oil, chloroform, etc., in place of xylene. Painter¹ has developed a method of substituting aniline oil for the higher concentrations of alcohol, replacing the aniline oil with methyl salicylate (oil of wintergreen) and passing from the latter into paraffin. This method does not harden wood provided the specimen is carried through very gradual changes. The several liquids diffuse so slowly, however, into half-inch cubes composed of xylem, cambium and phloem that the method is impractical for this material.

Mlle. Larbaud² has dehydrated and cleared with a mixture of ethyl and n-butyl alcohols. Butyl alcohol is soluble in paraffin in all proportions, but only 8.3 grams are soluble in 100 cc of water. A mixture of equal parts of ethyl and butyl alcohol, however, is

completely miscible with water. Larbaud based her technique upon the ethyl alcohol-xylene series: 30 per cent., 60 per cent., 80 per cent., 95 per cent., absolute alcohol, 2 pts. absolute alcohol-1 pt. xylene, 1 pt. absolute alcohol-2 pts. xylene, xylene. She shortened the above eight stages to six by using equal parts of ethyl and butyl for the alcohol of the first four stages followed by two changes of pure butyl alcohol.

Small cubes of wood with cambium and phloem attached require more gradual dehydration. The following series of mixtures of water, ethyl and butyl alcohol has been satisfactory:

Water	95-89-82-70-50-30
Ethyl alcohol.....	5-11-18-30-40-50
Butyl alcohol.....	0- 0- 0- 0-10-20

One hour is generally enough for each step except the last. The material should remain over night in this solution which contains a total of 70 per cent. alcohol. It should be emphasized here that alcohol not only dehydrates the tissue but also, as a powerful reducing agent, completes chrome fixation by reducing the chromate ion ($-\text{CrO}_4$) to the chromic (Cr^{+++}). Keeping the specimen in alcohol over night eliminates certain irregularities in the fixation images of various chromic compounds (Zirkle³).

The dehydration is completed by the stages:

Water	15- 5- 0- 0- 0
Ethyl alcohol.....	50-40-25- 0- 0
Butyl alcohol.....	35-55-75-100-100

An hour in each stage is generally sufficient except that the tissue should remain in the pure butyl alcohol until *all* the water is extracted.

As butyl alcohol dissolves solid paraffin extremely slowly, nothing is gained by placing chips of paraffin in the vial of alcohol that contains the specimen and by waiting for them to dissolve in the cold. A simple method is to fill a vial two thirds full of paraffin, let the paraffin harden and place the material to be embedded upon it. Cover the specimen with butyl alcohol and place the vial in the oven. As the paraffin melts, the tissue sinks. Butyl alcohol, being lighter than melted paraffin, does not sink with the specimen which consequently comes into intimate contact with almost pure paraffin. Two changes of paraffin are generally sufficient, the length of each change depending upon the size of the specimen.

In spite of the fact that butyl alcohol diffuses into paraffin more slowly than does xylene, it has several advantages over the latter as a clearing agent. Its specific gravity is less, being .810 at 20° C. compared with .881 for orthoxylene and .866 for metaxylene.

¹ *Anat. Record*, 27: 77-86, 1924.

² *Compt. Rend. Acad. Sci. Paris*, 172: 1317-1319, 1921.

³ *Protoplasma*, 4: 201-227, 1928; 5: 511-534, 1929.

It is slightly lighter than paraffin at the latter's melting-point and when the specimen sinks, as described earlier, it remains floating on the paraffin. Xylene, on the other hand, is heavier than melted paraffin and sinks with and surrounds the specimen. There is an additional advantage in the use of butyl alcohol as slight traces of it in the paraffin blocks do not render them crumbly as does a like amount of xylene.

Butyl alcohol does not harden wood, and its use makes the higher concentrations of ethyl alcohol unnecessary. Any wood that can be sectioned green may be sectioned in paraffin if dehydrated, cleared and embedded as described above. Thus it is possible to get thin, smooth sections of the soft cambium and phloem even if hard xylem elements occur in the slices. Woods as hard as hickory (*Carya ovata*) and

locust (*Robinia Pseudo-Acacia*) have been sectioned satisfactorily. The fine cytological details which can be investigated by this technique are destroyed by the prolonged hydrofluoric acid treatment, a necessary concomitant of the colloidin technique. However, butyl alcohol does not soften wood which has once been hardened by fixation, by drying, or by a too rapid dehydration, and all such material should be prepared for sectioning in other ways.

The simplicity of the butyl alcohol method, as pointed out by Larbaud, makes it preferable for other plant organs: buds, leaves, root-tips, etc. It is much quicker than the ethyl alcohol-xylene technique.

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SPECIAL ARTICLES

GEOLOGICAL EVENTS IN THE HISTORY OF THE INDIO HILLS AND THE SALTON BASIN, SOUTHERN CALIFORNIA

THE landward extension of the long depression occupied by the Gulf of California has experienced a very eventful history during Tertiary time. In the course of an investigation of the Indio Hills, now in progress, several facts of geologic significance have been discovered.

The trough, extending northwestward for nearly three hundred miles from the head of the gulf, was formerly known as the Colorado Desert. Its topographic subdivisions, in order from the gulf northwestward to its head at San Geronio Pass which leads into the coastal portion of Southern California, are the Colorado River delta or alluvial cone, Imperial Valley, Salton Sink or Salton Sea, and Coachella Valley. It is flanked on the west throughout much of its length by high mountains, rising to a maximum height of about 10,000 feet; it sinks to about 275 feet below sea-level beneath the recently formed freshwater Salton Sea. Tertiary marine and continental sedimentary formations outcrop at various places along the margins and bottom of the trough and yield clues to its origin and geological history.

The area of these deposits which we are studying, known as the Indio Hills, lies north of Indio, in central Riverside County. A similar neighboring area to the southeast, known as the Mecca Hills, is being investigated by Mr. Hampton Smith.

The Indio and Mecca Hills trend northwest-southeast, and lie end to end. Each of the two areas measures about twenty miles long by two to six miles wide, and together they extend most of the distance from the Salton Sea to the northwest end of the

Coachella Valley. They occupy a position parallel to and only slightly northeast of the median line of the broad depression. The hills rise about 1,000 feet above the plain and in the very arid climate exhibit a magnificent badland topography.

Our investigation is part of a broad program of Tertiary history studies initiated by Dr. John C. Merriam and sponsored by the Carnegie Institution of Washington.

1. We find that the upper part of the Indio Hills block is constituted of two formations. One of these is marine and is doubtless the correlative of the Carrizo formation, named by W. S. W. Kew¹ with type locality on Carrizo Creek, southwest of Salton Sea. It outcrops in the Indio Hills as small areas at several localities both east and west of the mouth of Thousand Palms Canyon and in the northern part of the hills. It consists of yellow clays with some sandstone and conglomerate. Its age was determined as upper Miocene by Ralph Arnold² and as lower Pliocene or younger by T. Wayland Vaughan.³ The formation, containing a fauna related to that living in the Gulf of California and very distinct from the west coast invertebrate assemblages of California, records an invasion of the Gulf of California over the Indio Hills block. This incursion has been determined by other workers to have extended almost to San Geronio Pass.

The second formation is several thousand feet thick and consists entirely of arid-climate, terrestrial de-

¹ "Tertiary Echinoids of the Carrizo Creek Region in the Colorado Desert," Univ. Calif. Publ., *Bull. Dept. Geol.*, 8: 2, 1914.

² U. S. Geol. Surv. Bull. 396, p. 44, 1909.

³ U. S. Geol. Surv. Prof. Pap. 98, p. 369, 1917.