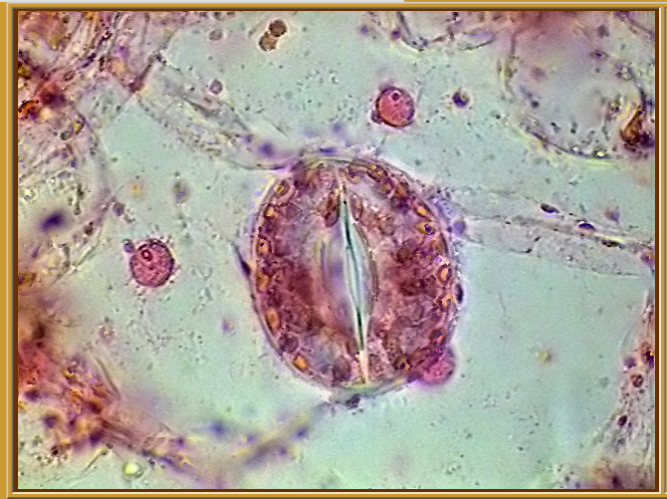
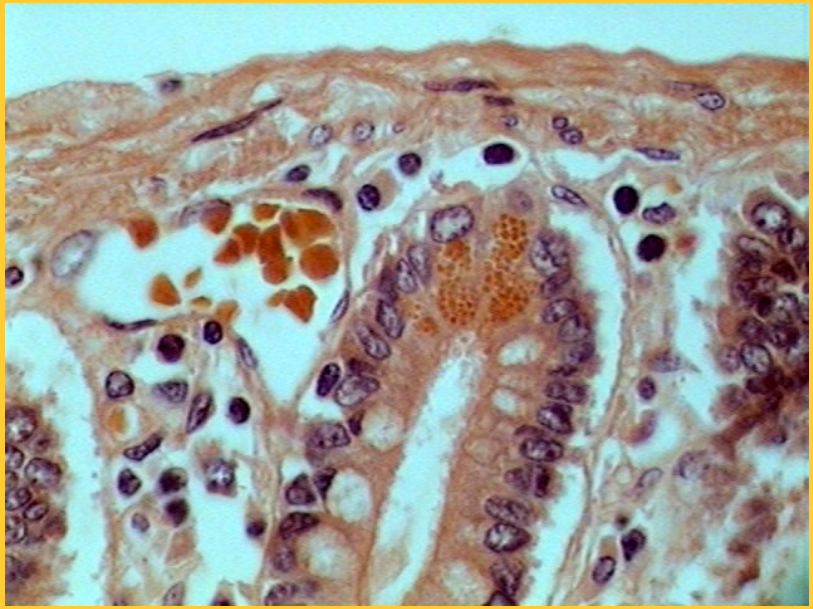


Eosin as a nuclear stain in botany and as an economical substitute for carmine in zoology

WALTER DIONI Cancún (Q.Roo) Mexico

Except where indicated to the contrary, the included images are personal pictures obtained with a digital camera of 0.4 Mpx. integrated into my National Optical DC3-163-P microscope equipped with planachromatic optics, (Ocular 10x, Objectives: x4 (NA 0.10), x10 (NA 0.25), x40 (NA 0.65) and x100 HI (NA 1.25)). The original ones have been captured at 640 x 480 px. and reduced or trimmed as was necessary to include them in this work. An entire picture's formatting, including mosaics of several pictures was made in Photo Paint. In each picture's legend, the objective whereupon it was taken is indicated, just as a suggestion of the power used because of the diferent sizes of each picture. A number of contrast devices (Rheinberg discs, darkfield discs, COL discs, and the Mathias arrow), could had been used to impart color, or relief to some images.

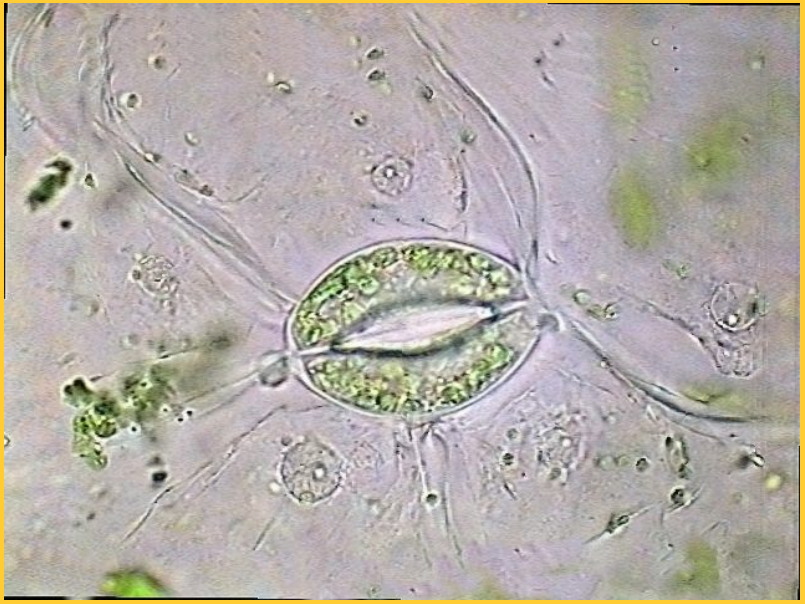
Eosin is an acidic aniline dye, which is considered to have a selective affinity for the cell cytoplasm. In contrast, one considers hematoxylin as a basic dye, which consequently has an affinity with the cellular acidic elements. As the nuclei are essentially formed by ribonucleic acids, hematoxylin is used to color them.



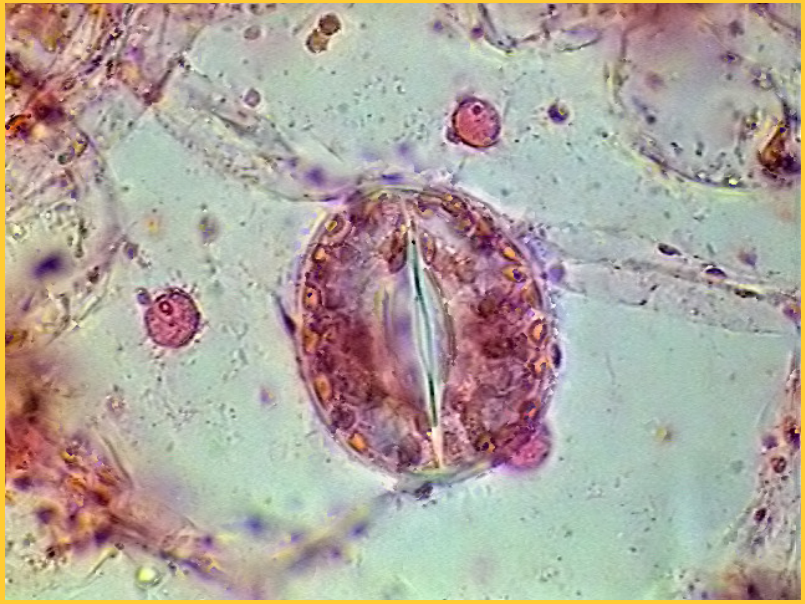
1 – Paneth glands, at the base of a Lieberkühn's Crypt. Histological section of human duodenum. Fixed in neutral formalin, paraffin embedded, 6 micron thickness microtome sections, stained with Harris Hematoxylin and Eosin. 40x planachromatic objective. Critical illumination, halogen light with didymium filter.

These two dyes are the basis of the universally used staining technique generally known as Hematoxylin-Eosin, which provides nuclei stained blue, (or almost black, according to the formula employed, see fig 1) and reddish cytoplasm.

The use of a commercial 2% eosin solution that was sent to me by Jean-Marie Cavanihac showed that in the foliar epithelium of *Aptenia cordifolia* the eosin strongly stained the nuclei (and nucleoli) as well as the chloroplasts, and much more slightly or not at all the cytoplasm.



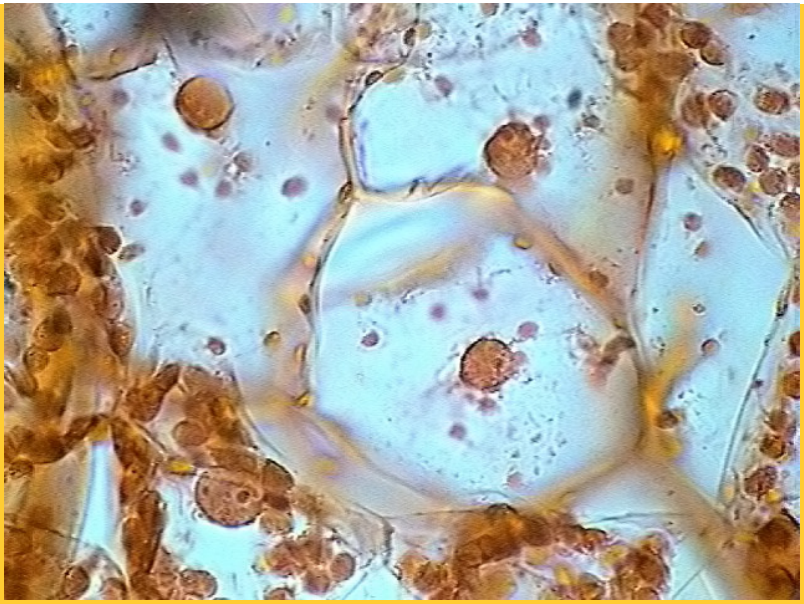
2 - Stoma in the lower epidermis of an *Aptenia cordifolia* leaf, unstained, mounted in glycerine 30%. Obj. x100.Two images stacked with CombineZ5.



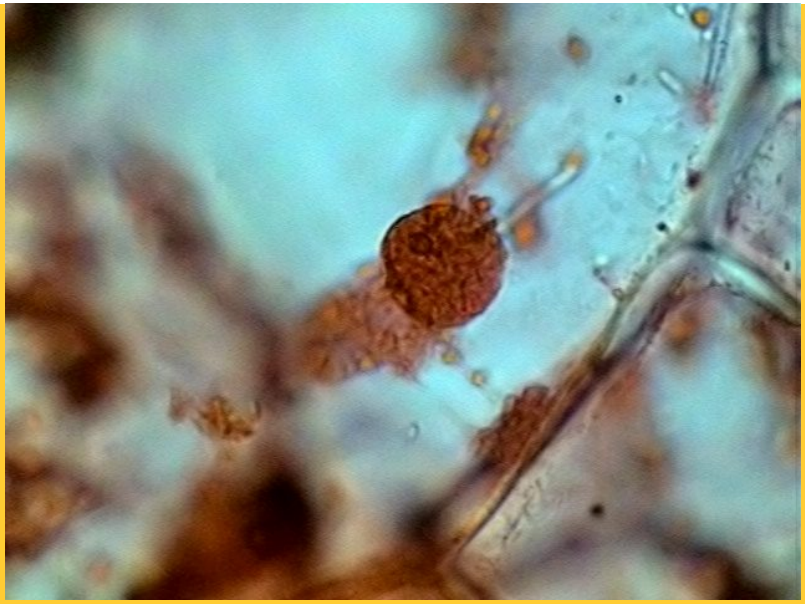
3 - Stoma of *Aptenia cordifolia*, fixed with AFA, stained with 1% eosin, mounted in glycerin with preliminary concentration. Obj. X100. Four images stacked with CombineZ5.

I have never known the use of eosin as a nuclear stain, and a very extended research through the World Wide Web did not provide any reference in this direction, largely reinforcing again the use of the eosin as a simple acidic dye.

In consultation with Jean-Marie Cavanihac he queried the existence of some additive in the commercial solution which could be responsible for such a behavior that one could consider "abnormal", and suggested trying some solutions directly prepared in the laboratory.



4 – Epidermic cells of *Aptenia* . Fixed in AFA, stained with 1% eosin, mounted in glycerin, after concentration. Obj. X100.



5 - Nucleus of a lacunar cell, top epidermis of the leaf of *Aptenia* . Fixed with AFA, stained with 1% eosin, mounted in glycerin after concentration. Obj. X100.

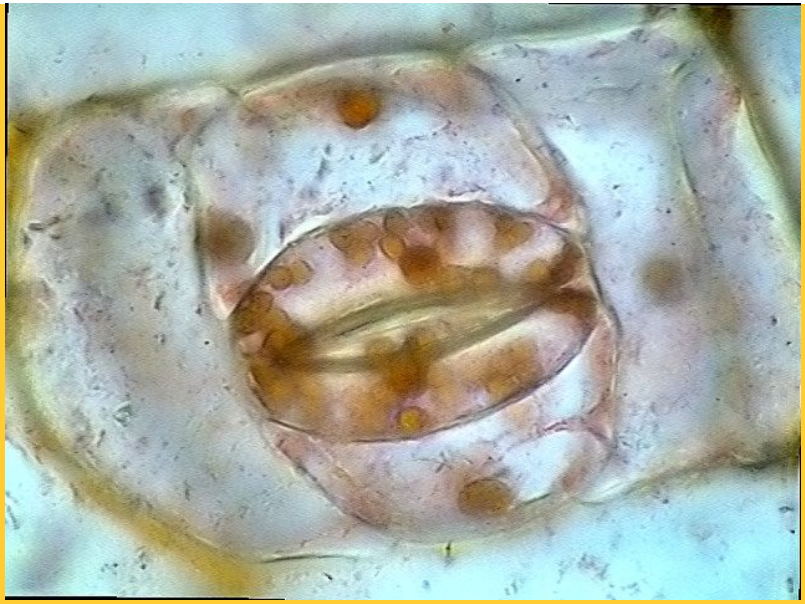
Consequently by using eosin in powder form, bought in an old pharmacy at Durango, I prepared two solutions of eosin:

1) In an ethanol-methanol mixture (MEM, ethanol 96%....70ml; methanol 85%....30 ml) and

2) Directly in distilled water.

Moreover I also used the European commercial solution.

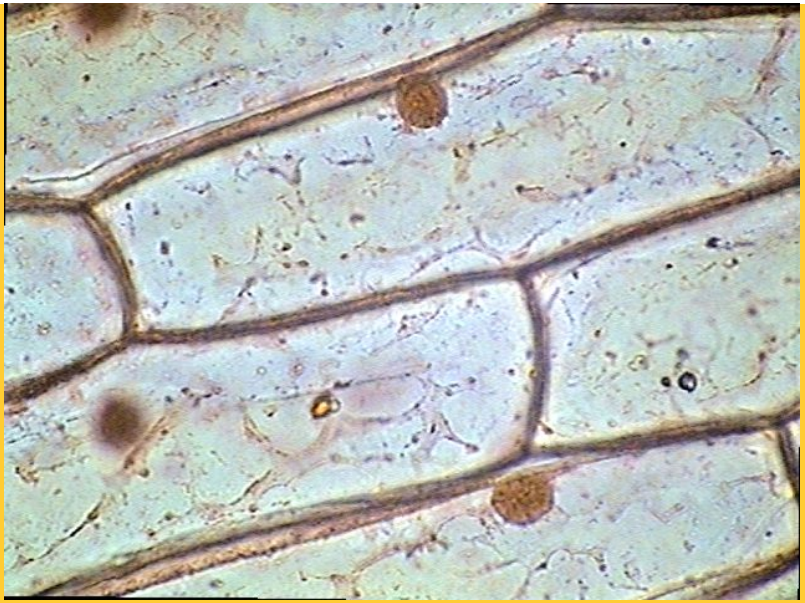
6 - *Rhoeo spatifolia* , stoma. Fresh epidermis, mounted in glycerin 30%. Obj. X40.



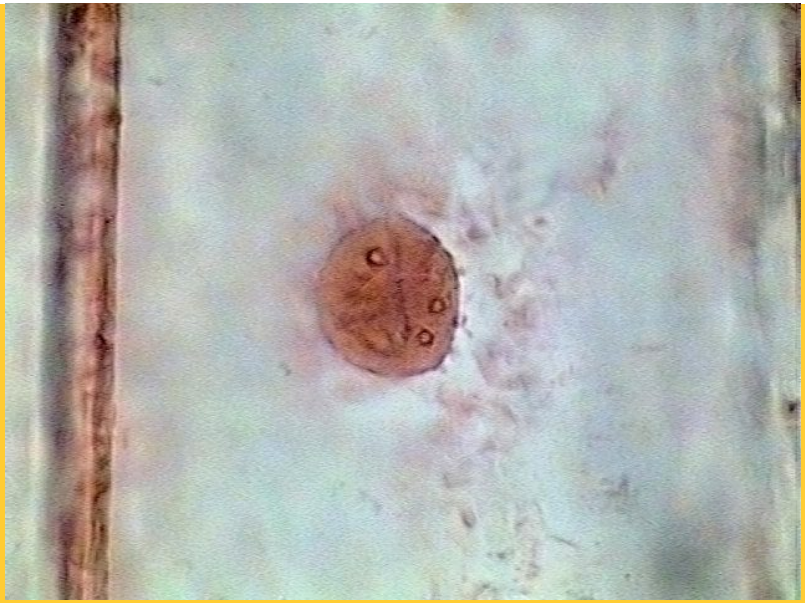
7 - Stoma, *Rhoeo* , epidermis fixed with AFA, stained with eosin 1%, mounted in glycerin 50%. Obj. X100. Three images stacked with CombineZ5.

Preparing solution in the alcohols proved to be difficult. Alcohol dissolved little of the dye and the solution seems to be a saturated one. The color was clear, transparent and yellowish. My eosin was definitely an "aqueous" eosin.

Water easily dissolved a larger quantity of eosin. The diluted solution showed at first the same color as the alcoholic solution, but, as it become concentrated, it turned a deep red as the commercial solution was.



8 - Cells from onion skin. Fixed in D1b; a new fixer with $ZnCl_2$. The fixation made evident the cytoplasm that surrounds the great central vacuole. Stained in 1% eosin, mounted in 50% glycerin. Obj. X40.



9 - Nucleus of a cell from the former preparation, showing the 3 nucleoli. Similar treatment as in the fig. 8. Obj. X100. Two focus levels stacked in Helicon Plus.

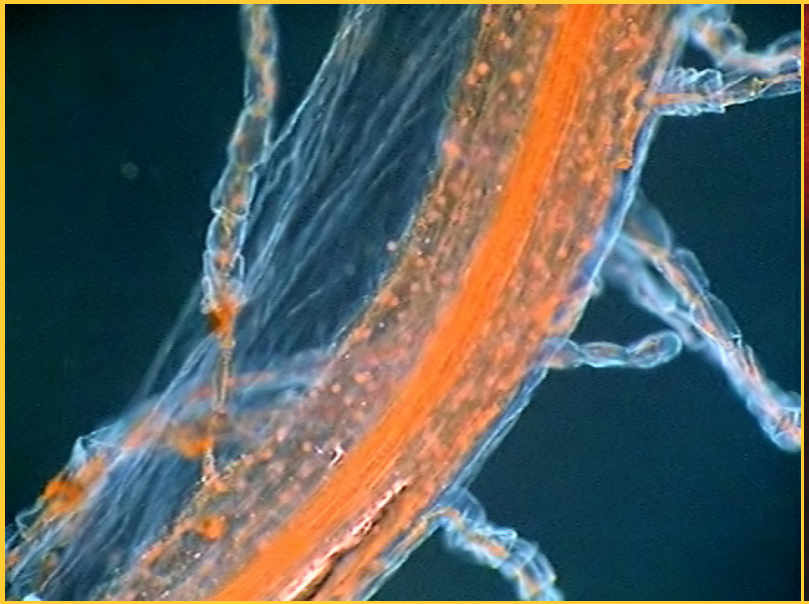
USES

1 – Foliar epithelia

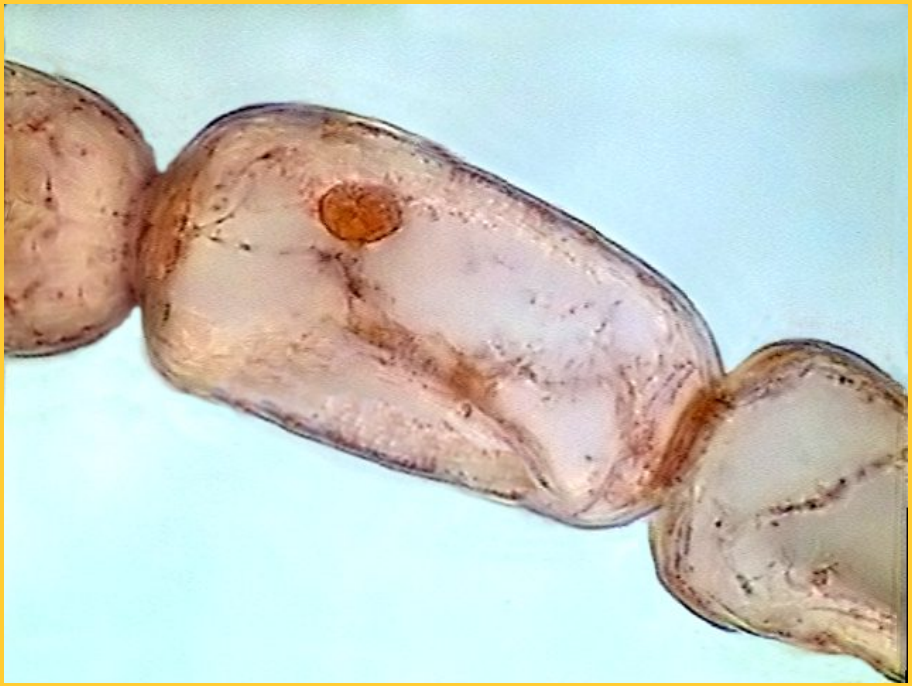
Three pieces of foliar epithelia from *Aptenia cordifolia* were fixed in AFA, (Alcohol, Formalin, Acetic Acid) for one hour. They were washed in two water changes, and one of them was submerged in one of each staining solution for 5-6 minutes.

Each epidermis was then carefully washed in two changes of water, until they did not lose more dye and they were then mounted between slide and coverslip in 50% glycerin.

The slides were manually compressed under a layer of absorbing paper to obtain quite a thin and readable preparation. They had been sealed with nail polish to improve their permanence.



10 - 11 - Stamen of *Tradescantia virginica* . Fixed in AFA, stained with 1% eosin. Mounted in 30% glycerin. In the lefthand picture one can see the central vessels, the stained nuclei of the cells, and some "stamen filaments". These filaments are excellent for showing the movement of the live parietal cytoplasm. Obj. X10. A filter W-Rh (Rheinberg with red and blue quadrants). On the right, the epidermis of the stamen with X40 objective, the treatment was explained in the preceding image.



12 - A cell of a staminal hair from a flower of *Rhoeo spathifolia* . Fixed in D1b, colored with 1% eosin, mounted in 30% glycerin. Obj. X100. Three focus levels were combined with Helicon Focus. The nucleus is suspended in the center of the cell, and it was captured by the median plane image.

Results

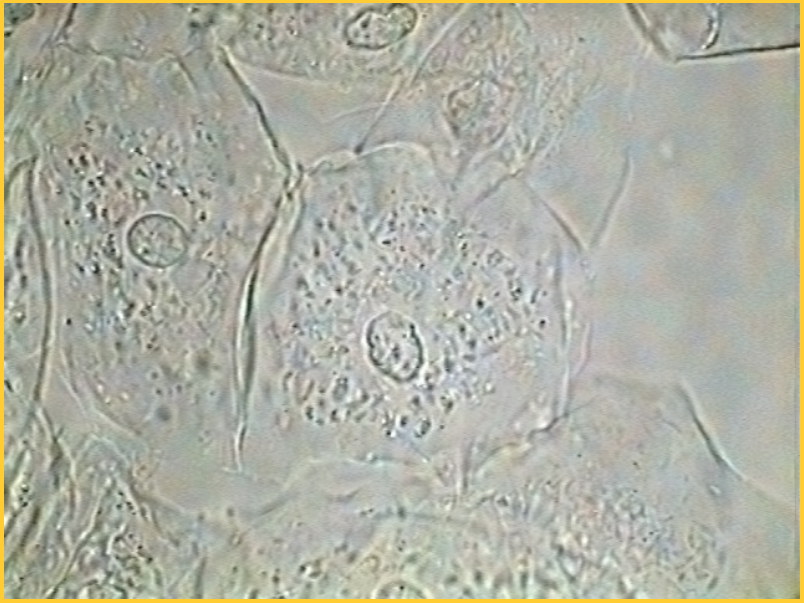
1) **European Commercial Eosin** : as in the preliminary test, nuclei and nucleoli, as well as the chloroplasts, were well colored, not therefore the cytoplasm.

2) **Aqueous Eosin** : The result is very similar to that of the commercial solution.

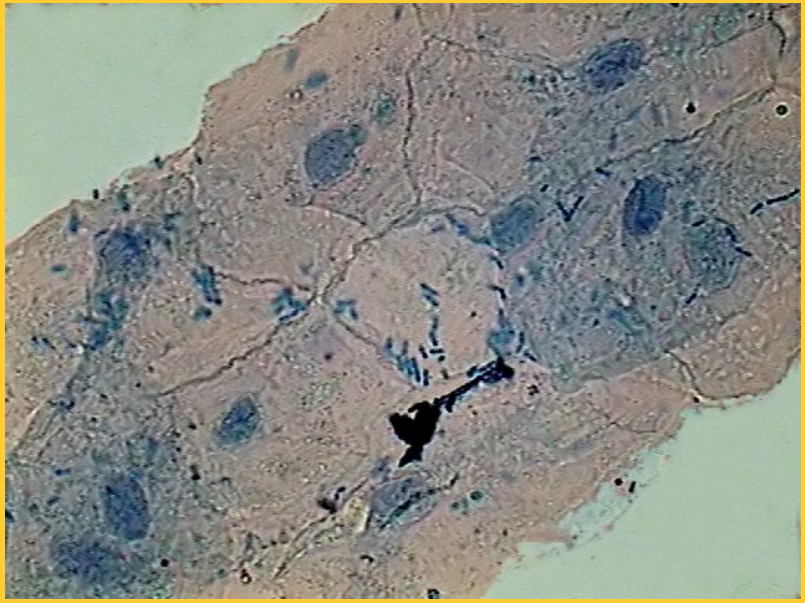
3) **Alcoholic Eosin** : in spite of its much clearer and yellowish color and the weak concentration of dissolved eosin, the result was very similar to the two preceding ones. Perhaps the nuclei are a little sharper and clearer than in the other two solutions. If the material supports an alcohol treatment this technique can save a lot of eosin.

2 – Gingival Epithelium

Cells obtained by softly scraping the interior of the cheek were mounted in diluted physiological solution 50/50 with aqueous eosin.



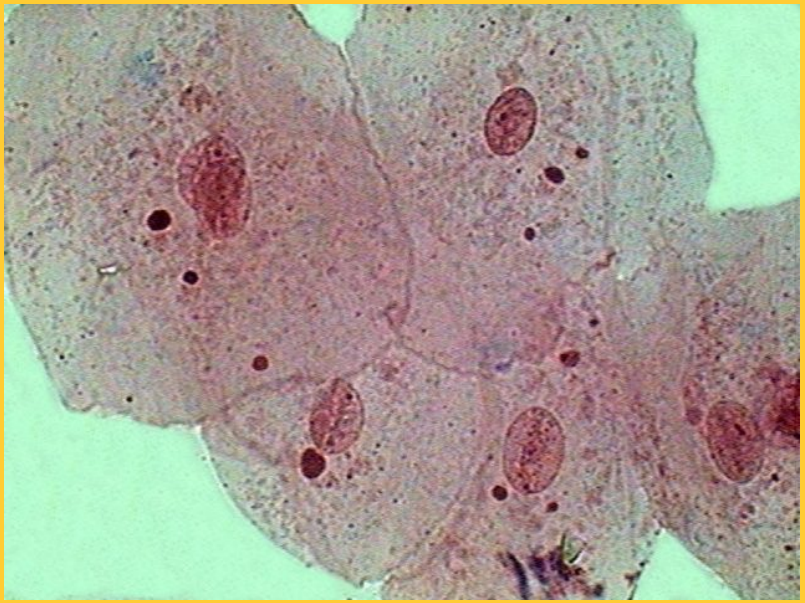
13 - Cells of the cheek, in physiological solution, without staining, bright field, X100 objective

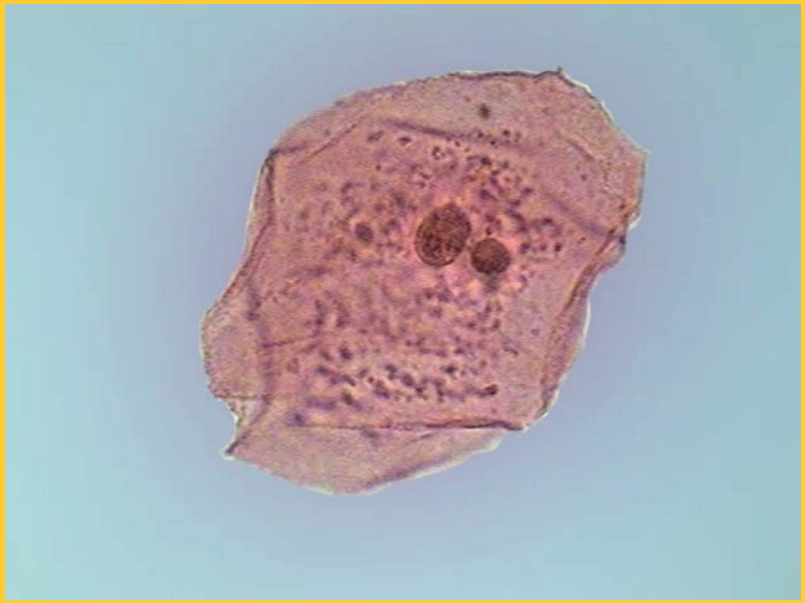


14 - Cells of the cheek mounted in water after staining with 1% methylene blue. Brightfield, X100

Results : The examination showed that the cells were suitably colored and that in a few seconds the nuclei and its nucleoli were quickly differentiated.

A parallel staining was carried out with methylene blue (the solution for aquarists) it also colored the nuclei, and the bacteria in the outside of the cells additionally, but the aspect is not so clear and clean as it was with the eosin.



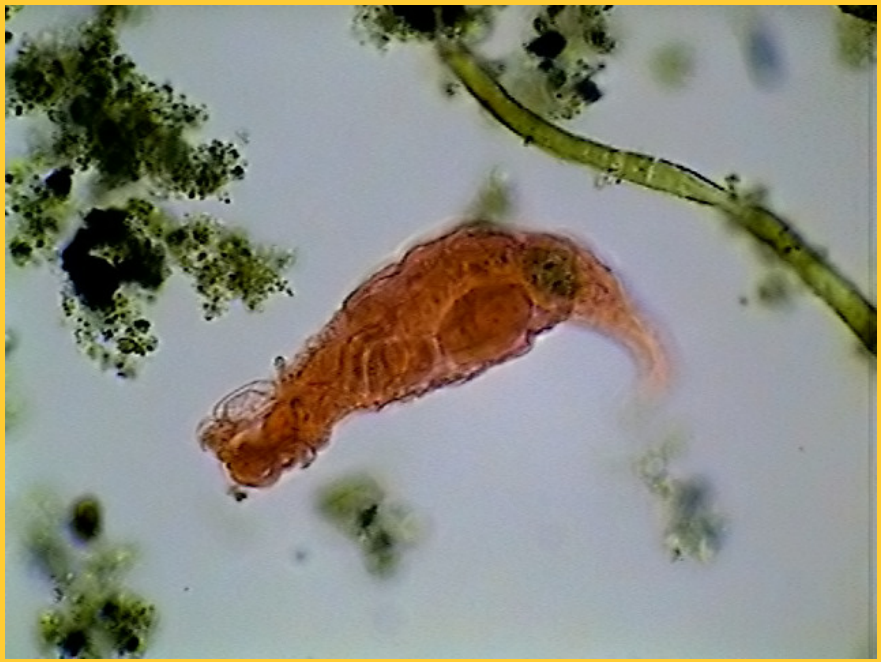


15 - 16 - Two images of cells of the cheek colored with 0.1% aqueous eosin. Those at left were mounted in a concentrated borax solution (sodium borate). At right a cell in water, four images treated by CombineZ5, the background was substituted with Photo Paint (X100 objective.)

Staining with eosin, and then with blue like a contrast stain, did not prove to be adequate. Blue was only partially fixed in the cytoplasm. The eosin dominated the staining and was not removed from the nuclei by the blue.

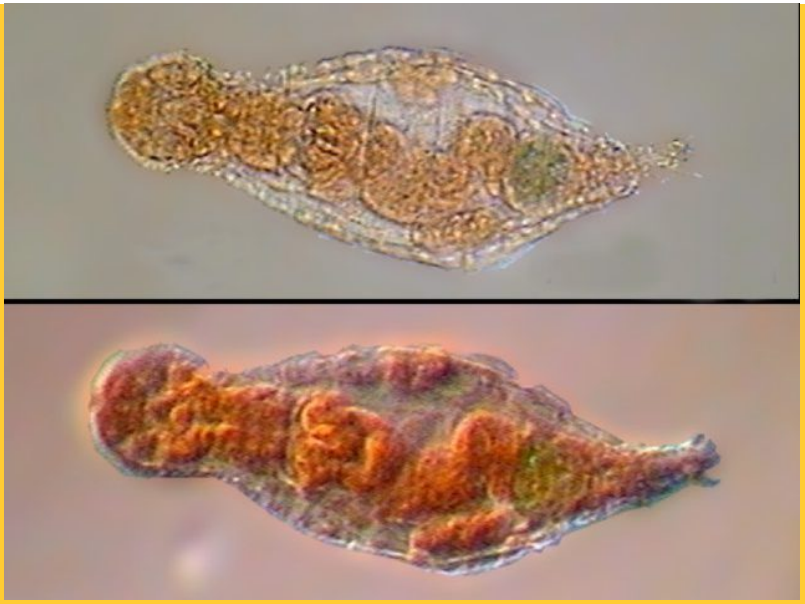
3 – Rotifers

A population of *Adineta sp.* was fixed by very hot water. Once the liquid was cold, 2% eosin was added drop by drop until a red, weak but definite color, was obtained. A small drop of the deposit was mixed with a similar drop of 50% glycerin, and it was covered with a coverslip aiming to obtain a very thin preparation.



17 - A species of *Adineta* , fixed with 60ºC hot water, stained with 0.5% eosin, mounted in 25% glycerin. Obj. X40. Detritus did not take up the dye, but the animals were strongly colored.

Results : the examination of this preparation (and other additional ones) showed that the rotifers are colored intensely enough, taking the dye in a diffuse manner in its tissues (it does not color the nuclei specifically) providing a sound differentiation of internal organization. The preparations look very similar to those of the Myers collection, colored with carmine, although the color is more orange.



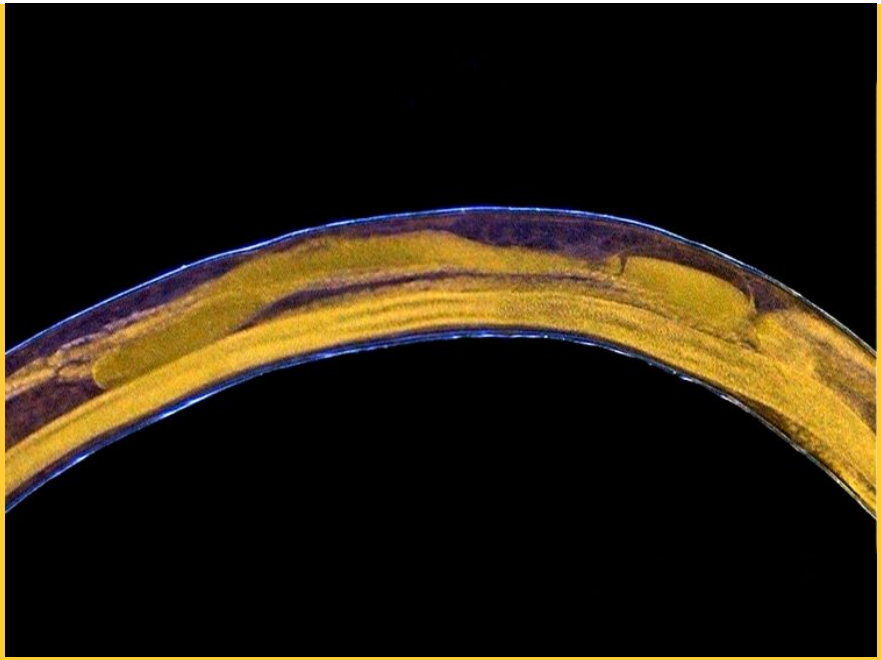
18 - A specimen of *Adineta* fixed by heat , colored with eosin and mounted in glycerin, upper image in bright field, lower image with oblique illumination

19 - Two examples of rotifers stained with carmine, and mounted in glycerin. Images taken from the database of Philadelphia ANS to compare with my results with eosin.

4 – Nematodes

Physaloptera squamata nematodes parasitic on the Cancun small lizard of the walls (*Anolis* sp.) fixed in 70% alcohol, were placed in a solution of 0.5% eosin for 5 minutes. The nematodes took a strong color. They were then passed to 15% glycerin, and after 20 minutes they were mounted in 30% glycerin.

Results : Contrary to what is recommended in the bibliography, where staining nematodes is considered very difficult, and where it is recommended to puncture the cuticle to allow a better absorption of the dye, the eosin almost immediately colored even very intensely the specimens of *Physaloptera squamata* used.



20 - A portion of a male *Physaloptera* , fixed with 70% alcohol, stained in eosin and mounted in glycerin. Photographed through a W-Rh blue-red filter. For better display of the image, I replaced the original background (blue dark) for a black one. In the lower half of the worm one can see its intestine, above it one just see the testicles, and the seminal vesicle.

The contrast filters can be used precisely as with fresh material, although they give other effects of color and they provide interesting additional possibilities for effects of oblique illumination.

Important details, related to these experiments are: 1) to use an aqueous fixer, 70% alcohol deformed the animals a little; 2) to wash out with water and to probably pass to a very diluted eosin (0.1 or 0.2% would be enough); 3) to supervise staining, transferring often to clean water, and to stop it, washing in 5% acetic acid (vinegar); 4) to eliminate the acetic acid by washing in pure water; 5) to pass to 10% glycerin and to let concentrate until approx. 50%; 5) to work up out of Glycerin, mounting in Glycerin Jelly or in PVA-G.

If one uses a more concentrated staining solution and over colors the specimens, it is possible to regress staining by fading it with 0.1% hydrochloric acid. The reversal must be supervised at X40 since it delays only a few minutes. Stop the regression by washing with abundant water. Mount as before.

Conclusions:

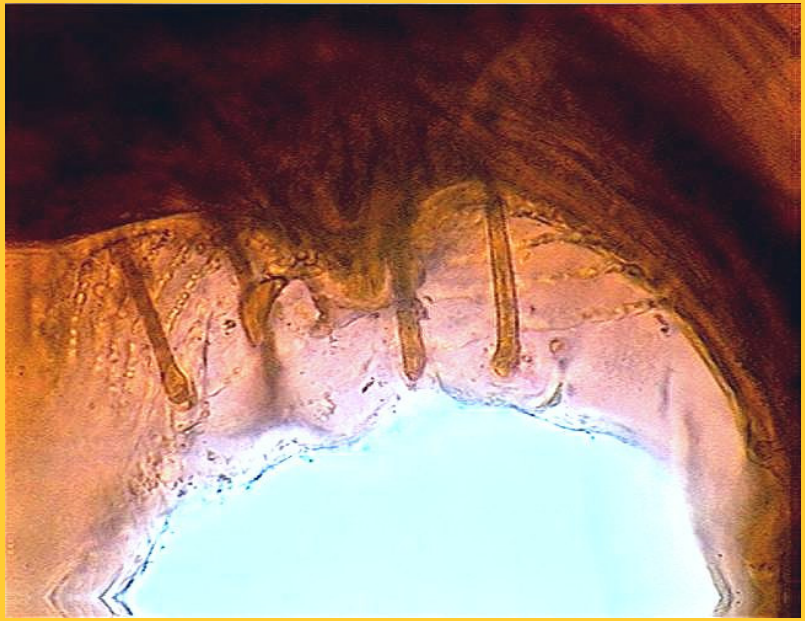
In the case of the vegetable epidermal staining, eosin offers an important advantage over the examination of fresh products, without coloring. Nuclei, nucleoli, chloroplasts and cytoplasm are better differentiated. The aspect is also more attractive and if the materials were fixed beforehand (with AFA or GALA) and one treats them by adequate methods, permanent preparations can be made.

The staining and glycerin mounting of rotifers definitely gave better results than those obtained by mounting unstained individuals.

For the nematodes mounted in glycerin after clarifying them beforehand, some tissues are distinguished by a greater absorption of the dye or a different shade of the color. Body organs are thus clearly differentiated.

Contrast discs, and the Rheinberg method especially, made it possible to improve even more the topographic study while offering very interesting possibilities of color and oblique illumination.

21 - Caudal end of a male *Physaloptera* . One sees by transparency the caudal allules which are used to embrace the female, and four long genital glands located on each allule. Objective X10. Brightfield. Background replaced with Photo Paint.



22 - Objective X40. The glands, and, between second and third, the characteristic genital spicules. Similar treatment for these two images as in the first one. Brightfield.

In *Physaloptera* the analysis of the structure of the caudal wings, the nervous system and the excretory system, was much better with staining, than in the individual without color. Also some glandular structures are better seen.

However in the case of small nematodes, generally a preparation mounted in glycerin without any stains (the standard method of nematologists) has also a good definition of organography and it also allows an intelligent use of the contrast discs.

Followed-up by image processing in a photo-processor can offer in some cases and for some details even better results than staining.

The preceding tests indicate that it can be profitable to try additional groups of invertebrates (Temnocephalida, Digenea, Monogenea, Cestodaria, Turbellaria, Micro-oligochaeta, etc.) since eosin is a cheap stain which is obtained rather easily. It could be an economic substitute of the traditional carmine …. at least for amateurs.

PERMANENCE : Four months after the tests, the stain is maintained with the same intensity and still better definition than when they were freshly made.

Comments to the author, Walter Dioni , are welcomed.

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