dielectric constant. The osmic acid and Altmann techniques, indicate that such a substance, a lipoid, is localized in the wall of the vesicle.

The shape of the vesicle is in constant flux, diverticula being slowly formed, retracted, or fused together. On several occasions spherical bodies have been seen to form from these diverticula. One of the bodies became highly refractile and meanwhile its color changed to the orange-amber of the fat droplet in the base of the cell.

All observations indicate that the vesicle is an extremely irregular sac, having a lipoidal wall, and filled with a fluid of low viscosity. Although the composition of the wall and the relation to the nucleus suggest that the element is the Golgi vacuome, such a conclusion seems premature until further investigations have been made.

7003 C

Pinacyanol as a Histological Stain.

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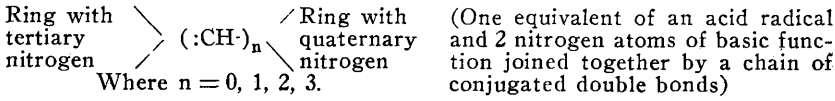
Pinacyanol is a dye belonging to the cyanine group. During the last 10 years great advances have been made, chiefly by Mills[1] and his coworkers, in the preparation and elucidation of the chemical constitution and photographic properties of the cyanine dyes. There are about 37 different types of cyanine dyes known.

The cyanine dyes have the peculiar property of conferring extra sensitiveness on silver halides, which are only sensitive to the blue and violet regions of the spectrum. If the proper dyes are added to the emulsion, or if the films are bathed in the solution, they become extremely sensitive to red, yellow, orange, green and to the invisible infra-red portions of the spectrum. This sensitizing property is probably due to the following characteristic molecular grouping present in all sensitizing cyanine dyes:



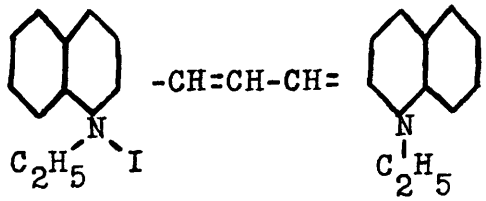
The cyanine dyes are strongly basic dyes, the monoacid salts are

[1] Doja, M. Q., *Chem. Rev.*, 1932, **11**, 3.

colored while the diacid salts are colorless. The chemistry of the sensitizing action is unknown. However, we do know that the dye is adsorbed by the silver halide.

The cyanine dyes* available commercially are: Orthochrome T (sensitizer for green), Pinacyanol (sensitizer for red), Kryptocyanine (sensitizer for extreme red), Dicyanine A (sensitizer for infra-red), Neocyanine (sensitizer for infra-red) and Quinoline blue.† These dyes, with the exception of quinoline blue‡ have not been used for histological purposes. The staining properties were investigated for various tissues. Pinacyanol was the most satisfactory, not only as a polychromatic stain cat exogen but also for its quick staining action. Naphtocyanol and Dicyanine A were less satisfactory but may be used for special purposes. Kryptocyanine and Orthochrome T stained diffusely without differentiation. Neocyanine had no staining effect at all.

Pinacyanol has the following formula:



It is formed by the condensation of quinaldine ethiodide on itself in the presence of formaldehyde and contains 3 methylenyl groups between the two rings. Pinacyanol iodide crystallizes from alcohol in the form of fine blue-green needles. The alcoholic solution is dichroitic; in reflected light it is blue and in transmitted light it is red. Dissolved in pyridin it becomes a pure blue with slight dichroism and on adding water, the color changes to violet-red.

For histological purposes 0.5 gm. of pinacyanol are dissolved in 100 cc. absolute ethyl or methyl alcohol. However, this solution

* These dyes may be obtained from the Eastman Kodak Co., Rochester, N. Y.

† Quinoline blue (chinolinblau) may be obtained from Dr. Gruebler, Leipzig.

‡ Quinoline blue (Greville Williams, 1856), the oldest cyanine dye, was first used by Certes (*American Microscopic Journal* No. 3, 1883) in very dilute solutions 1:100,000 to 1:500,000 for staining infusoria. When used in these dilutions it will stain only the fat granules. Ranvier advocated a very dilute alcoholic solution of the dye for staining fresh as well as preserved objects. Vivante used the dye for staining decalcified bone; Walker for staining vegetable fat (*Int. Monatsschrift f. Anat.*, 1882, **9**); Golenkin for the detection of free or bound iodine (*Bull. Soc. Nat. Moscou*, 1899); Zimmermann for staining cellulose membranes.

may be further diluted with alcohol in proportion 1 :3 or 1 :5 according to the required staining intensity. The solution will keep indefinitely if placed in clean, glass-stoppered bottles, preferably pyrex glass, and protected from the light. The aqueous solution is not stable and deteriorates in a short time.

Pinacyanol gives the best results with either native or fixed frozen sections. Formol or Formol-Mueller fixation gives equally good results. It may be used in paraffin or celloidin embedded material, but the brilliancy and differential staining of the various tissue elements is entirely lost. For staining frozen sections, the section is mounted on a slide and covered with a few drops of the alcoholic staining solution which is allowed to remain for from 5 to 10 seconds; the section is then floated from the slide into distilled water, washed for about 15 seconds, remounted, and embedded in C. P. glycerin.

Pinacyanol is a nuclear as well as a protoplasmic stain. The chromatin ranges in color from dark blue to cobalt violet and is very distinctly differentiated. The protoplasm is stained in various shades of purple. It further differentiates connective tissue from elastic and muscle tissue. The connective tissue is stained eosin-red, the elastic tissue a deep black-violet and the muscle tissue in different shades, ranging from blue-violet to purple. The neutrophile and eosinophile granules are not stained while the granulo-plasma of the plasma cells is stained a brilliant carmine-red, standing out prominently from all other inflammatory cells. The hemoglobin remains unstained while the neutral fat is either colorless or presents a faint bluish-violet hue. The various lipoids are stained a deep bluish-violet to deep purple. Pinacyanol is also an excellent stain for amyloid, which is almost instantaneously stained a distinct carmine-red without any further differentiation. The hemosiderin is stained a brilliant orange-yellow.

Glycerin seems to intensify the staining effect. The intensity of the color of the granulo-plasma of the plasma cells and of the elastic fibers increases considerably after the sections are mounted in glycerin. The permanence of the staining effect depends greatly on the kind of tissue and the state of preservation. Frozen sections of tissue fixed in Formol or Formol-Mueller have so far retained their color for a period of 7 months. Stained frozen sections can not be dehydrated and embedded in balsam. Alcohol decolorizes practically all of the protoplasmic substances while the chromatin remains more or less intensely stained.

Pinacyanol offers the opportunity for the most detailed cell studies, not only from a morphological but from a histo-chemical standpoint.