

## WRIGTH'S STAIN—MODIFIED.

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This polychromic stain, originally known as Romanowsky's stain, has had many modifications. In principle, they all depend upon the loose chemical combination of eosin (tetrabromofluorescin) with methylene-blue, these dyes staining not only as units but acting together when properly combined. The two in solution in the same solvent, therefore, contain at least three compounds, each of which colors the various structures of the specimen, selectively.

This particular modification yields an eosinated methylene-blue solution which will not only produce a strong differential stain but simplifies the technic of both the preparation of the stain and the staining of specimens, and it gives more uniform results.

*Solution No. 1.*

Methylene-blue (Merck) .....	5.0
Sodium bicarbonate .....	2.5
Distilled water .....	500.0

Place the methylene-blue and the sodium bicarbonate in a 1000 cc. flask, add the distilled water and connect the flask with a twelve inch reflux Liebig condenser. Transfer this apparatus to a water-bath and heat for two hours, and then cool.

*Solution No. 2.*

Tetrabromofluorescin (Merck) .....	4.0
Water, distilled .....	250.0

Dissolve the tetrabromofluorescin in the distilled water by agitation or stirring.

After solution number one has cooled to 20° C., add solution number two slowly and with frequent agitation. Collect the precipitate thus formed on well-wetted filter. While it is still wet dissolve it in 60 cc. of hot methyl alcohol (acetone free), and again filter. Evaporate this solution to one-half its volume and set aside for the eosinated methylene-blue to crystallize. Collect the crystals thus formed and after they have been dried spontaneously, transfer them to a well-stoppered, amber-colored bottle.

## THE STAIN.

Eosinated methylene-blue .....	.1
Methyl alcohol .....	100.0

Dissolve by agitation.

The methyl alcohol should be acetone free and the eosinated methylene-blue should be made by the above process.

This stain, like all polychromic stains, decomposes rather rapidly and should therefore be made in small quantities. Amber bottles will retard the decomposition somewhat.

## METHOD OF STAINING.

Pour the stain over the surface of the specimen until it covers it and allow to remain for from fifteen seconds to one minute; this period of time varying with the thickness of the specimen. This serves to fix the film to the glass as well as to stain it, so it is not necessary or desirable to pass the preparation through the flame. Now add distilled water, drop by drop, until a slight metallic

scum is produced on the surface of the stain. At this point a precipitation occurs and the real staining takes place which takes from five to ten minutes. Next wash in distilled water for several minutes or until the thin parts of the specimen have acquired a yellowish tint. The differentiation may frequently be brought out more plainly by washing longer.

If the blood-smear, as an example, stains a uniform deep-blue, distilled water will bring about a differentiation, removing the blue from the acidophilic parts, leaving them well stained with an eosin color.

The differentiation produced by this stain is as perfect as can be obtained by any of the eosinated methylene-blue combinations, and is being used with a great deal of success on blood-smears, demonstration of bacteria in cellular exudates, pus, etc.

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### DETERIORATION OF NITROGLYCERIN TABLETS.

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Although within the past five or six years several prominent pharmaceutical chemists have expressed the opinion that nitroglycerin tablets are a stable preparation, there are still some who believe in their instability and that the deterioration is chiefly due to volatilization.

In 1907, Bernegau<sup>1</sup> stated, that "the loss of nitroglycerin appears to be in the granulation process, and that the tablets themselves are fairly constant" and Dohme<sup>2</sup> in the same year stated "that observations made in his laboratory appear to indicate that tablets of nitroglycerin do not deteriorate perceptibly in course of time." In 1908, Gane and Webster<sup>3</sup> referred to "nitroglycerin as a stable compound" and Edmunds and Roth<sup>4</sup> find that "contrary to the common opinion, nitroglycerin tablets do not deteriorate greatly with age." Again 1909, Dohme<sup>5</sup> comments upon a "comprehensive investigation of the deterioration of the tablets, that disprove the claim that these tablets deteriorate rapidly" and very recently Vanderkleed<sup>6</sup> stated that "nitroglycerin tablets when properly made are to be classified among the more stable products, \* \* \* and the tablets will remain unchanged or practically so, for a considerable length of time."

The last comment was in reply to an editorial article referring to the very unstable nature of nitroylcerin tablets due to loss by evaporation.

Until the phenoldisulphonic acid method was proposed by Scoville<sup>7</sup> and its modification by the Bureau of Chemistry,<sup>8</sup> and also the May method,<sup>9</sup> there was no very reliable method for accurately determining nitroglycerin in tablets. We have found the modified Scoville method to be a very practical and reliable method.

The purpose of this paper is to report the results of several experiments and while not covering a very long period or many samples, we believe the figures are quite sufficient to demonstrate the stable nature of nitroglycerin tablets.

Samples of 0.01 (1/100) and 0.02 (1/50) grain hypodermic tablets, which were assayed on April 12, 1912, having been made sometime previous, were set aside on a laboratory shelf in ordinary cork stoppered glass tubes of