THE EFFECT OF PYRIDOXINE ON THE URINARY EXCRETION OF A NEW FLUORESCENT SUBSTANCE[1]

RECENT reports[2, 3] have indicated that there are two fluorescent substances in the urines of normal individuals that are related to the level of nutrition of nicotinic acid. These have been named F_1 and F_2. The latter is absent in the urines of pellagrins, but readily appears after adequate nicotinic acid therapy. While investigating the possible correlation of this phenomena with the anti-pellagra and anti-black-tongue activities of nicotinic acid and related pyridine derivatives, the present authors observed a marked increase of fluorescence in the F_1 fraction following the ingestion of pyridoxine hydrochloride by normal and pellagrous patients. A dose of 100 mg of this vitamin is followed by a four- to ten-fold increase of a bluish-purple fluorescence during the first four-hour period as compared to the preceding control period. The average values of fluorescence in Najjar-Wood units for the control and test periods are 4 and 32, respectively. The urinary substance responsible for the increased fluorescence resembles F_1 in its adsorption on permutit ("Decalso"), elution with 25 per cent. potassium chloride solution and extraction from the eluate with isobutanol, but differs, however, from F_1 in that it is extracted from only the untreated eluate. *In vitro* experiments with pyridoxine either alone or incubated for four hours with urine at pH 5.0 failed to demonstrate the presence of the unknown substance. We believe, therefore, that this unknown urinary constituent is a new entity and perhaps of significance in the intermediary metabolism of pyridoxine. Until its relationship to F_1 and F_2 is known, we hesitate to propose a name for its description. The details of studies, now in progress, on its physical and chemical nature will be reported elsewhere.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A NEW TECHNIC FOR STAINING VAGINAL SMEARS: III, A SINGLE DIFFERENTIAL STAIN

Two previous communications to this journal[1, 2] have described modifications of the Masson trichrome stain, adapting it to the staining of vaginal smears. Among the advantages of this technic for the interpretation of the vaginal smear are its specificity for the cornified elements and the addition of a sequence of color changes to the cytological alterations which follow the endogenous elaboration, or the administration, of the reproductive hormones. Although this staining method is simple and rapid, the number of solutions required renders it somewhat cumbersome for general clinical use. A single differential stain would be more likely to encourage the wider use of the vaginal smear as a diagnostic index and as a guide for the therapeutic use of the reproductive hormones.

The purpose of this note is to describe a single differential staining solution, based on the technic previously developed and retaining its most useful features. In it are incorporated all the components of the original staining method with the exception of hematoxylin. It provides a sharp differentiation between cornified and non-cornified elements. The former stain a brilliant orange-red; the latter take on a green stain which is deeper in the younger cells, and paler in those more advanced. The staining is delicate and reveals cytoplasmic and nuclear details very clearly. Other constituents, such as leucocytes, erythrocytes, bacteria and spermatozoa, are satisfactorily differentiated.

This composition of the Single Differential Stain (S3) is as follows:

ethyl alcohol (50 per cent.)	100 cc
Biebrich Scarlet (water sol.)	0.5 gms
Orange G	0.25 "
Fast Green FCF	0.075 "
phosphotungstic acid c.p.	0.5 "
phosphomolybdic acid c.p.	0.5 "
glacial acetic acid	1.0 cc

All the dyes are domestic and can be obtained from the National Aniline and Chemical Company. The solution should not be used until all the ingredients have dissolved completely.

The vaginal smears are prepared and stained as follows:

1. Aspirate the vaginal secretion by means of a dry pipette with rubber bulb attached, and expel onto a glass slide.
2. Fix, while wet, in equal parts of ether and 95 per cent. alcohol. Fixation for 1 or 2 minutes is adequate.
3. Stain for approximately 1 minute in Solution S3.
4. Carry through 70 per cent., 95 per cent. and absolute

[1] Acknowledgement is made of aid from the John and Mary Markle Foundation.
[2] V. A. Najjar and R. W. Wood, *Proc. Soc. Exp. Biol. and Med.*, 44: 386, 1940.
[3] V. A. Najjar and L. E. Holt, SCIENCE, 93: 20, 1941.
[1] SCIENCE, 91: 321, 1940.
[2] SCIENCE, 91: 579, 1940.

alcohol, dipping slide 10 times in each solution.
5. Clear in xylol and mount in damar.

In preparing the smear, it is important not to allow the secretion to dry before fixation, as drying alters the morphology and the staining properties of the cells. Very thick smears are also undesirable, since it is difficult to remove the excess stain from the thick areas except by prolonged washing in the alcohols. A thick secretion should be spread out thinly with the edge of the pipette, taking care to avoid drying in so doing. Most reliance can be placed in the histological picture in the thinner areas.

When single slides are stained, it is most economical and convenient to deliver the stain from a dropping bottle. If the portion of the slide containing the smear is marked off with a china-marking pencil, one or two drops of stain generally suffice. It is economical to drain the slide thoroughly and wipe off the back with a cloth or paper towel after each solution, thereby prolonging the dehydrating properties of the alcoholic solutions. The absolute alcohol can be eliminated, by blotting the slide thoroughly after rinsing in 95 per cent. alcohol, and then going directly to xylol. All solutions should be well stoppered when not in use. The use of coverslips can be avoided by using isobutyl merthacrylate polymer P–5 (du Pont de Nemours and Co. Ammonia Dept., Wilmington, Del.) instead of damar. This is dissolved in xylol to yield a solution of proper consistency for mounting. After the slide is cleared in xylol, a few drops of the solution are placed on the slide to form a protective film over the smear. The slide can be examined at once, and then allowed to dry in air before filing.

While most investigators may prefer to use the complete trichrome stain previously described for research purposes, the present technic should fulfil most of the clinical requirements for a rapid simple differential stain for vaginal smears.

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A DEVICE FOR THE PREPARATION OF HYDROSULFITE SOLUTIONS FOR GAS ANALYSIS

A DISADVANTAGE in the use of sodium hydrosulfite solutions for the rapid absorption of oxygen in gas analysis, as suggested by Van Slyke and co-workers,[1] is the need for frequent renewals of the solution. This drawback could be partially overcome if a method were used by which a considerable proportion of the hydrosulfite is not oxidized by the air during the preparation of the solution. This may be accomplished by a simple device which has been used in this laboratory for several years.

This consists of a glass tube 14 mm inside diameter and 195 mm long (volume, 30 cc), constricted at one end to a small glass stopcock. Below the stopcock, attached by means of a rubber connection and inserted into the stopcock tubing, is a 250 mm length of 3 mm outside diameter glass tubing. This tubing is to reach to the bottom of the gas apparatus container for the oxygen-absorbing solution. At the bottom of the larger tubing is a wad of fine, glass wool for filtering the solution. Twenty-five cc of 2 N KOH solution is poured into the tube, and somewhat more than ½ of the 15 gm of hydrosulfite is added. The tube is stoppered and shaken a few times. The solution is then run into the container, and a 2 cm layer of liquid petrolatum is simultaneously poured on to the surface. The second 25 cc of KOH solution and the remainder of the hydrosulfite are quickly added in a similar manner, and the absorbing solution is ready for use.

The useful oxygen-absorbing capacity of the hydrosulfite solution is greatly increased by this easy and rapid method of preparation.

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