gonelline,[4] we found that on a nicotinic acid-free diet F_2 excretion parallels closely the excretion of trigonelline (Table I). Removal of protein from the diet caused a sharp drop in the excretion of both F_2 and trigonelline. The rabbit, which is known not to methylate nicotinic acid to trigonelline,[5] excretes no F_2 after a dose of 250 mg of nicotinamide.

TABLE I

TRIGONELLINE AND F_2 EXCRETION IN RATS

	Trigonelline	F_2	*Diet*
Rat	/day	Units per day	
1	97	32	23 per cent. protein
2	148	136	23 per cent. protein
3	227	288	23 per cent. protein
	31	66	Protein free
4	433	695	23 per cent. protein
	100	201	Protein free

N-methyl nicotinamide and F_2 were found to be adsorbed on and eluted from permutit under the same conditions from urine, and from pure solutions.

Both are extractable by butanol from alkaline KCl solutions and not from neutral or acid solutions. The fluorescence intensity of both is much greater in butanol than in aqueous KCl solution. Colorless aqueous solutions of both, when treated with dilute KOH at ordinary temperatures, become yellow and are reversibly decolorized on acidification. Butanol extracts of the yellow alkaline solution, under a Wood screened ultraviolet light, show green fluorescence which changes reversibly to blue on acidification. The above green fluorescence increases to a maximum in about 20 minutes. Both substances when heated with strong alkali also yield yellow solutions which are not decolorized on acidification. Butanol extracts of these yellow alkaline solutions show blue fluorescence which disappears on acidification and is restored on alkalinization. Hydrolysis of both substances in dilute aqueous HCl or KOH results in a greatly decreased fluorescence in their butanol extracts. This type of hydrolysis is known to convert the amide to the acid, or the N-methyl nicotinamide to trigonelline. Trigonelline also shows a very weak fluorescence under the above conditions.

Since the usual procedure for estimating trigonelline in urine[6] involves its hydrolysis to nicotinic acid with strong alkali in presence of ammonia (or urea), and since this treatment also converts both F_2 and the methyl nicotinamide to nicotinic acid, it is obvious that the previously published values for trigonelline in urine include substance F_2 in addition to true trigonelline.

Thus a normal adult who ingested 200 mg of N-methyl nicotinamide excreted in the urine in 48 hours F_2 equivalent to 55 mg of the original compound, as measured by its fluorescence, plus 85 mg of trigonelline.

From the urine of a normal adult, excreted in 20 hours after the ingestion of 750 mg of nicotinamide, we isolated a crystalline product as the picrate salt. The melting point of this product was 189.5° C (uncorr.) as compared with the melting point of the picrate of synthetic methyl nicotinamide which was identical, 189.5°, and the mixed melting point was unchanged. The picrates of the natural and of the synthetic products decomposed in dilute acid and after removal of the picric acid with ether, yielded quantitatively the identical amount of fluorescence in the ultraviolet, when measured in the usual way for F_2.

F_2 thus appears to be a N-methyl nicotinamide or a labile precursor which yields this compound in the course of isolation. Further work on the chemical identification of this compound and on its metabolic behavior is in progress.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

POLYVINYL ALCOHOL: A MEDIUM FOR MOUNTING AND CLEARING BIOLOGICAL SPECIMENS[1]

LUBKIN and Carsten[2] have recently reported on the use of polyvinyl alcohol, or PVA, in a method for the elimination of dehydration in histological technique. It is the purpose of the present note to report the use of PVA as a medium for the mounting and clearing of biological specimens.

PVA, a synthetic polymer of vinyl alcohol, is available through the E. I. du Pont de Nemours Company, R. & H. Chemical Department, Niagara Falls, New York. It may be purchased at low cost (less than $1.00 a pound) in pound lots or more.

[4] Jesse W. Huff and W. A. Perlzweig, *Jour. Biol. Chem.*, 142: No 1, 401, January, 1942.

[5] Y. Komori and Y. Sendju, *Jour. Biochem.*, 6: 163, 1926.

[1] The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Division of The Rockefeller Foundation.

[2] SCIENCE, 95: 633, 1942.

[6] William A. Perlzweig, Edward D. Levy and Herbert P. Sarett, *Jour. Biol. Chem.*, 136: No. 3, 729, December, 1940.

[7] Nutrition Foundation fellow. This study was also aided in part by grants from the John and Mary R. Markle Foundation and the Duke University Research Council.

The substance is obtained either as a light white powder or as amorphous granular material. It is soluble or dispersible in water, making a clear solution, syrupy in consistency. The viscosity of this solution can be varied by the amount of material added. PVA is not soluble in any of the ordinary fat solvents. The watery solution, upon drying, leaves a transparent, tough, thin film which adheres closely to a grease-free surface. This film is resistant to dampness, water (except immersion for a period of several hours), alcohol, ether, xylol, acetone and other solvents.

The stock solution is prepared by adding PVA (Grade RH-349) powder slowly to cold water, stirring it in thoroughly. The powder goes into solution with difficulty, but the process can be hastened by heating in a steam bath until the solution becomes about as viscous as thick molasses. At this time the solution appears milky, owing to the inclusion of small air bubbles. Upon further heating, or if left to stand for several hours, it becomes water clear. Any undissolved material can be strained off. The stock can be stored and preserved indefinitely and diluted with water to any consistency desired.

Several uses have been found for this stock solution. Giemsa stained blood films, both thin blood films and thick drops, have been preserved by spreading a thin film of stock solution over the stained portion and drying this quickly. Slight fading takes place before the solution dries completely, but after it has dried, such slides, placed even in direct sunlight or under ultra-violet lamp, will not lose any further color. The blood films are covered by a tough, thick film and after examination with oil the oil can be wiped off with a cloth with no possibility of damaging the smear. Slides examined two years after preservation by this method are still in excellent condition.

The stock solution has also been found useful for the examination of mosquito larvae. Live larvae are placed on two or three drops upon a slide. The larvae are quite firmly held by the viscous solution and can be examined most minutely for as long as half an hour. Upon completion of examination the larvae can be washed off into a container of water and soon recover. Smaller living organisms such as trematode cercariae may be placed in a drop of the solution and covered with a cover glass, following which they can be examined under oil immersion for twenty or thirty minutes before they die. This method also works well for detailed examination of motile microorganisms.

A clearing and mounting medium has been prepared by a modification of the original lacto-phenol medium which has the composition of glycerine 60 per cent., phenol 20 per cent., lactic acid 20 per cent. by volume. The original medium clears certain types of material satisfactorily but remains liquid and has to be sealed in, a very time-consuming procedure. We have prepared a somewhat similar preparation which we call polyvinyl-lacto-phenol: PVA stock solution 56 per cent., phenol 22 per cent., lactic acid 22 per cent. by volume. The resulting medium readily clears small objects removed directly from aqueous solution. Mosquito larvae can be satisfactorily mounted by dropping live larvae on to slides, draining off the water, adding 4 to 6 drops of PVA lacto-phenol and covering with a cover glass. More mounting medium should be added if necessary, so that a small amount of the medium flows out on all sides of the cover slip. In two days the cover slip will be cemented firmly in place and the larvae well cleared. In some cases it is necessary to add more medium later if it recedes under the cover slip while drying. Such mounts preserve all details necessary for identification.

A very satisfactory technique has been worked out for the mounting of the terminalia of male mosquitoes. The terminalia, after being cleared in 10 per cent. potassium hydroxide and stained with acid fuchsin, are dissected in a drop of PVA lacto-phenol. The viscosity of the solution aids dissection. The dissected parts can be transferred to a very small drop of solution on a clean slide and arranged as desired. The slide can then be dried in an incubator or on a hot plate so that the parts are firmly held in place in the dried film. A drop of mounting medium is then placed on a cover slip and the preparation covered. This method is rapid and simple.

The method also offers a quick and satisfactory means of mounting pollen grains for measurement and detailed examination and gives a permanent mount.

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