treated with iproniazid respond similarly.

There is some evidence to indicate that the lethal effects of epinephrine are mediated in the central nervous system, at least in part (*9*). Penetration into the hypothalamus (*10*), as a result of prolonged high levels in blood, and subsequent potentiation of the amines centrally is also a possible explanation.

These results tend to support the hypothesis that iproniazid may, in part, exert its effects by potentiating epinephrine and norepinephrine (*11*).

J. L. Borowitz
William C. North
Pharmacology Department, Northwestern University Medical School, Chicago, Illinois

References and Notes

1. P. A. Shore, *J. Clin. Exptl. Psychopathol.* 19, suppl. 1, 56 (1958).
2. A. Sjoerdsma *et al., Proc. Soc. Exptl. Biol. Med.* 89, 36 (1955).
3. A. Carlsson, M. Lindquist, T. Magnusson, *Nature* 180, 1200 (1957).
4. A. N. Davison *et al., Experientia* 13, 329 (1957); E. A. Zeller and J. Barsky, *Proc. Soc. Exptl. Biol. Med.* 81, 459 (1952).
5. E. C. Griesemer, thesis, Northwestern University Medical School (1955).
6. A. Luisada, *Arch. Exptl. Pathol. Pharmakol. Naunyn-Schmiedeberg's* 132, 313 (1928); *Medicine* 19, 475 (1940).
7. E. C. Griesemer and J. A. Wells, *J. Pharmacol. Exptl. Therap.* 116, 282 (1956); J. Axelrod, *Pharmacol. Revs.* 11, 402 (1959).
8. E. A. Zeller, J. Barsky, E. R. Berman, *J. Biol. Chem.* 214, 267 (1955).
9. B. Cassen, W. Gutfriend, M. Moody, *Proc. Soc. Exptl. Biol. Med.* 93, 251 (1956).
10. H. Weil-Malherbe, J. Axelrod, R. Tomchick, *Science* 129, 1226 (1959).
11. This investigation was supported by a fellowship (MF-7841) from the Division of Research Grants of the National Institutes of Health, Public Health Service. We wish to thank Hoffman-LaRoche for their generous supply of iproniazid. This work was presented at the Federation Proceedings in Atlantic City, 16 Apr. 1959.

25 May 1959

Acrylamide Gel as a Supporting Medium for Zone Electrophoresis

Abstract. Acrylamide polymerized in buffer solutions forms a stable, flexible, transparent gel which is useful in zone electrophoresis.

Many different forms of stabilizing media have been proposed for use in "zone electrophoresis." Since Smithies described the starch gel technique (*1*), the use of gelling agents to stabilize the migration medium has received increasing attention (*2*). This report describes a new, commercially available, synthetic gelling agent which has many advantages over previously described agents for electrophoresis.

Cyanogum 41 is a product of American Cyanamid Co. The following paragraph is quoted from the technical information supplied by the manufacturer:

"Cyanogum 41 Gelling Agent is a mixture of two organic monomers—acrylamide and N,N^1-methylenebisacrylamide—in proportions which produce stiff gels from dilute aqueous solutions when properly catalyzed. The process by which the gels are formed is a polymerization-cross-linking reaction."

Gels can be formed with various buffers according to the directions supplied with the material. The precaution must be observed of covering the surface of the solution so as to exclude oxygen when casting thin sheets or films of the gel. This is easily accomplished by floating a sheet of nonwettable plastic on the surface of the monomer solution during the polymerization period.

We have found that 3- to 5-percent Cyanogum in acid or alkaline buffers (0.3 to 0.01*M*) produces a satisfactory gel for use in electrophoresis. The gel is optically clear and colorless. The optical density of a 5-percent gel 1 mm thick increases slowly from < 0.01 at 650 mμ to 0.04 at 325 mμ, rising sharply between 300 mμ and a peak at about 260 mμ (optical density, 0.41), then rising to an end absorption. The gel is flexible and elastic, stable, and completely insoluble in water, once it has formed. At concentrations of 5 percent and more it is strong enough to support its own weight but is somewhat brittle unless a plasticizer, such as glycerol (5 percent) is included in the formulation.

The rate of migration of serum albumin in 0.1*M* tris buffer of *p*H 9, gelled with 5-percent Cyanogum, is approximately 1.2 cm/hr at a field strength of 10 volt/cm. A serum pattern can be developed in this medium in 8 hours, fixed in dilute acetic acid, and stained with bromphenol blue, as in any of the current standard procedures. Washing in dilute acetic acid turns the gel (which takes up the stain) a pale yellow and leaves the protein pattern dark blue. The pattern is sharper than in other commonly used media. Several of the globulin zones are separated into subcomponents.

Hemoglobin in the same medium migrates at approximately one-half the rate of serum albumin. There is less tailing of the hemoglobin bands than is seen on paper strips. There is a marked decrease in the width of individual bands as compared with starch gel patterns. The hemoglobin patterns resemble those seen on agar gel, with some improvement in resolving power. The clarity of the Cyanogum gel permits direct measurement of the pattern by transmitted-light photometry through the gel, without staining.

A particularly convenient way of preserving the electrophoretic patterns, whether stained or not, is to allow the gel to dry out completely. In drying the gel shrinks uniformly in all dimensions, producing a thin, flexible, transparent, celluloid-like film which preserves the original pattern relationships. This film can be rehydrated to its original dimensions by soaking in water.

Samuel Raymond
Lewis Weintraub*
Pepper Laboratory, University of Pennsylvania, Philadelphia

References

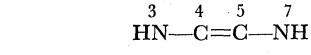
1. O. Smithies, *Biochem. J.* 61, 629 (1955).
2. C. A. J. Goldberg, *Clin. Chem.* 4, 484 (1958).
* Present address: University Hospital, Ann Arbor, Mich.

21 May 1959

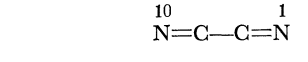
Mechanism of Enzymatic Oxidation of Purines

Abstract. Tautomeric forms of 2-hydroxypurine, in which the structure has been fixed by introduction of an N-methyl group, are oxidized differently by xanthine oxidase. The 1-methyl derivative is attacked at position 8 and the 3-methyl derivative at carbon atom 6. These observations indicate that 2-hydroxypurine itself reacts in a tautomeric form, corresponding to the structure of its 1-methyl derivative.

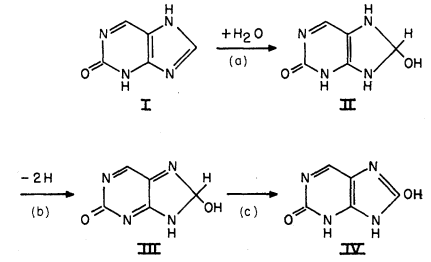
The fact that 2-hydroxypurine (I) is converted by mammalian xanthine oxidase (XO) into 2,8-dihydroxypurine (IV) has been interpreted previously in the following way (*1*): (a) structure I adds a molecule of water across the CH=N double bond of the imidazole ring; (b) the hydrated form (II) transfers two hydrogen atoms from its central portion, comprising



directly to the corresponding dienic system of the flavin nucleus in xanthine oxidase, represented by the grouping



(c) the intermediate III undergoes a hydride shift to give IV. In this scheme, the order of steps (i) and (ii) may be reversed.



Recent experiments on the enzymatic oxidation of 8-azapurines (*2*) and pteridines (*3*), however, make the above interpretation improbable and require a new approach to the mechanistic prob-