trode catheter whereby hydrogen potential measurements may be made simultaneously at various points and where blood samples and pressure measurements can be taken at the same time (*3*).

LELAND C. CLARK, JR.
L. M. BARGERON, JR.
Departments of Surgery and Pediatrics, University of Alabama Medical Center, Birmingham

References and Notes

1. L. C. Clark, Jr., and G. A. Misrahy, *Proc. Intern. Physiol. Congr., 20th Congr., Brussels* (1956), abstr. No. 177; G. A. Misrahy and L. C. Clark, Jr., *ibid.* (1956), abstr. No. 650.
2. L. C. Clark, Jr., *Trans. Am. Soc. Artificial Internal Organs* 2, 41 (1956).
3. This research was supported by U.S. Public Health Service grants H-3109 and H-2602 (C-2). We express our appreciation for the interest and encouragement of Dr. Champ Lyons.

Note added in proof: Since this report was submitted for publication, a large number of diagnostic catheterizations have been completed, by means of the hydrogen electrode catheter; these are described in a report in *Surgery* (in press).

13 April 1959

Potentiation of Epinephrine and Norepinephrine by Iproniazid

Abstract. The effects of pretreatment with iproniazid on the toxicity and cataract-producing ability of epinephrine and norepinephrine were studied. The epinephrine and norepinephrine were administered in such a way that a slow, prolonged rate of absorption was achieved. Under these conditions, the lethality and cataract-producing ability of these amines were shown to be significantly enhanced by the action of iproniazid.

To explain the psychic effects of iproniazid, it has been postulated that this substance potentiates the effects of certain physiological amines by inhibiting monamine oxidase (*1*). Although potentiation of serotonin (*2*) and dihydroxyphenylalanine (*3*) has been produced by preadministration of iproniazid, significant potentiation of the effects of epinephrine and norepinephrine by iproniazid has not been shown previously.

To demonstrate potentiation by iproniazid, a slow, prolonged rate of absorption of epinephrine and norepinephrine was attained in three ways: by subcutaneous administration, by repeated intraperitoneal injection, and by slow intravenous perfusion of the amines. At more rapid rates of absorption, no potentiation could be shown. For example, we were not able to show potentiation of single intraperitoneal doses of norepinephrine by iproniazid in rats, nor were we able to show a significant difference in the amount of norepinephrine required to kill rats perfused intravenously at a relatively rapid rate.

In the experiments described below, doses of iproniazid reported to inhibit completely monamine oxidase were used (*4*). Mice routinely received 100 mg/kg, and rats, 50 mg/kg. In each case, enough time was allowed for the iproniazid to inhibit the monamine oxidase before the catechol amines were administered. Doubling the dose of iproniazid in mice did not further potentiate the effects of the amines.

The results given in Table 1 show that iproniazid potentiates the toxicity and cataract-producing ability of subcutaneously administered epinephrine and norepinephrine. Both multiple small doses and a single larger dose of these amines were effective in demonstrating the potentiation. Griesemer had found incidentally that a single subcutaneous dose of 0.5 mg of epinephrine per kilogram which killed none of 15 controls, killed 21 of 41 rabbits that had been injected with 50 mg of iproniazid per kilogram 12 hours and 2 hours prior to the administration of epinephrine (*5*).

Using a total of 130 rats, we could show no potentiation by iproniazid of epinephrine given in single doses intraperitoneally. The results of the studies of subcutaneously administered amines prompted us to administer the norepinephrine in divided doses intraperitoneally to obtain a more prolonged rate of absorption. One-half milligram of norepinephrine was given every 15 minutes for 13 doses to rats pretreated with iproniazid and to control rats pretreated with saline. Thirteen of 20 rats pretreated with iproniazid and four of 20 controls died of this treatment ($p = < 0.01$).

In the studies in which the catechol amines were administered subcutaneously and intraperitoneally, the potentiation by iproniazid could have been due to the fact that the iproniazid prevented the amines from being destroyed before they were absorbed from the injection site. To test this possibility, the amines were infused intravenously at a slow rate. Unanesthetized rats were used, to avoid the effect of drugs which depress the central nervous system, which have been shown to antagonize the toxicity of epinephrine (*6*). The jugular veins of the rats were cannulated under light ether anesthesia, and an hour was allowed for complete recovery from the anesthetic. The weights of the animals in the two groups were identical. The animals were perfused with DL-norepinephrine bitartrate at a rate of 1 mg/cm^3 per 85 minutes. The rats pretreated with iproniazid died after having received 0.73 ± 0.66 mg, the controls, after having received 4.66 ± 1.74 mg ($p = <0.01$). The heightened sensitivity of rats pretreated with iproniazid to intravenously administered norepinephrine indicates that the potentiation seen in the studies in which the amines were administered subcutaneously and intraperitoneally was not due solely to a difference in the amount of intact amine available for absorption.

Seven controls and seven rats pretreated with iproniazid, when perfused with 1.5 mg of DL-norepinephrine per cubic centimeter per 85 minutes failed to show a significant difference in response, although, on the average, less of the amine was needed to kill the animals pretreated with iproniazid (0.83 ± 0.44 mg, as opposed to 2.0 ± 1.33 mg).

It has been shown that iproniazid profoundly alters the metabolism of epinephrine and norepinephrine, presumably by inhibiting monamine oxidase (*7*). Such inhibition provides a possible explanation for the potentiation of these catechol amines by iproniazid. It may be that the amines must be absorbed slowly in order to show the potentiation because the monamine oxidase system slowly deaminates the amines in vivo, as it does in vitro (*8*). At faster rates of absorption the monamine oxidase system may have little opportunity to inactivate any of the dose administered before a toxic level is reached; therefore, control animals and animals pre-

Table 1. Effects of iproniazid on the incidence of mortality and of cataract produced by catechol amines given subcutaneously.

Drug and No. of doses*	Total dose (mg/kg)	Species	No. dead at 24 hours per No. used		No. with cataract per No. of survivors at 4 hours	
			Animals pretreated with iproniazid	Controls	Animals pretreated with iproniazid	Controls
Epinephrine, 4	8	Mouse	17/20	5/20 †	7/8	5/17 ‡
Epinephrine, 6	6	Rat	18/18	9/18 †	4/6	0/16 ‡
Norepinephrine, 5	20	Rat	5/8	0/8 ‡	5/8	0/8 ‡
Norepinephrine, 1	12.5	Rat	10/10	4/10 ‡		

* Interval between doses, ½ hour. † $p < 0.01$. ‡ $p < 0.05$.

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

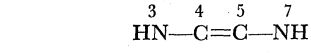
1. O. Smithies, *Biochem. J.* 61, 629 (1955).
2. C. A. J. Goldberg, *Clin. Chem.* 4, 484 (1958).
* Present address: University Hospital, Ann Arbor, Mich.

21 May 1959

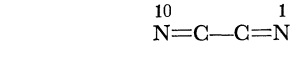
Mechanism of Enzymatic Oxidation of Purines

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.

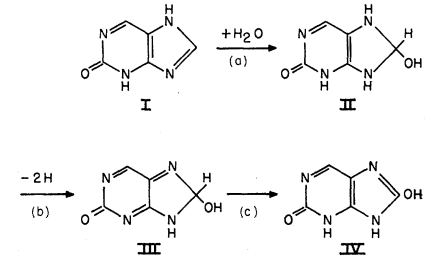
The fact that 2-hydroxypurine (I) is converted by mammalian xanthine oxidase (XO) into 2,8-dihydroxypurine (IV) has been interpreted previously in the following way (*1*): (a) structure I adds a molecule of water across the CH=N double bond of the imidazole ring; (b) the hydrated form (II) transfers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