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*).

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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.

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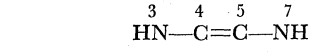
21 May 1959

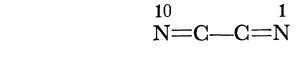
Mechanism of Enzymatic Oxidation of Purines

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.

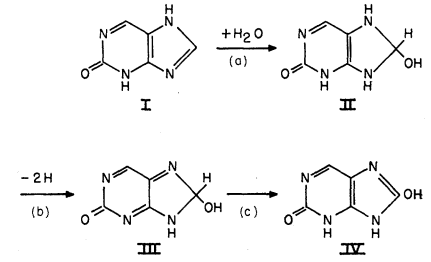
The fact that 2-hydroxypurine (I) is converted by mammalian xanthine oxidase (XO) into 2,8-dihydroxypurine (IV) has been interpreted previously in the following way (*1*): (a) structure I adds a molecule of water across the CH=N double bond of the imidazole ring; (b) the hydrated form (II) transfers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



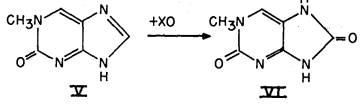
Recent experiments on the enzymatic oxidation of 8-azapurines (*2*) and pteridines (*3*), however, make the above interpretation improbable and require a new approach to the mechanistic prob-

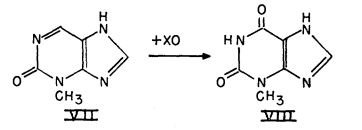
Table 1. Physical properties of 2-hydroxypurine and its derivatives.

Substance	$\lambda_{max.}$ at *p*H 8.0 (mμ)	R_f* acid solvent	R_f* basic solvent	Fluorescence†	Relative rate of oxidation‡ (xanthine = 100)
2-Hydroxypurine (*4*)	314	0.27	0.24	Blue	16.0
2,8-Dihydroxypurine (*4*)	304	0.28	0.28	Blue-violet	0.2
1,2-Dihydro-1-methyl-2-oxopurine (V)	318	0.36	0.30	Blue	180.0
1,2-Dihydro-1-methyl-2-oxo-8-hydroxypurine (*5*)	311	0.34	0.38	Sky-blue	Not attacked
2,3-Dihydro-2-oxo-3-methylpurine (VII)	315	0.51	0.39	Blue	100.0
2,3-Dihydro-2-oxo-3-methyl-8-hydroxypurine (*5*)	320	0.43	0.42	Blue	2.1
3-Methylxanthine	272	0.43	0.45	Black-violet	Not attacked

* Descending method; the following solvents were used for development: Acid solvent: 95 percent ethanol, 85 ml; glacial acetic acid, 5 ml; water, 10 ml. Basic solvent: 95 percent ethanol, 70 ml; pyridine, 20 ml; water, 10 ml.
† Fluorescence was observed with the aid of a Mineralight ultraviolet lamp, which emits light of λ about 255 mμ.
‡ All substrates were used at a concentration of 6 to 7 × $10^{-5}M$.

lem of purine oxidation in general. In order to determine the "active" form of a substrate entering into reaction with the prosthetic group of the enzyme, we have examined two isomeric monomethyl derivatives of I, in which a single tautomeric structure of the latter has become fixed. It was found that 1,2-dihydro-1-methyl-2-oxopurine (V) is oxidized in position 8 and thus resembles I. On the other hand, 2,3-dihydro-2-oxo-3-methylpurine (VII) is converted by xanthine oxidase into 3-methylxanthine. In both cases, identification of the oxidation product was facilitated by the fact that the enzymatic reaction does not proceed beyond the first step. Comparison of the accumulating end products with synthetic materials by paper chromatography and ultraviolet absorption spectra establishes their identity beyond doubt (see Table 1).





These results suggest that I combines with xanthine oxidase in a tautomeric form, corresponding to V. The latter is the fastest reacting substrate of xanthine oxidase yet found, for it is attacked almost twice as rapidly as xanthine. This leads to the conclusion that the pathway of oxidation of purines is determined not so much by the intrinsic polarity of a substrate as by the polarity of the specific enzyme-substrate complex. The active surface may attract preferentially a single structure out of a mixture of tautomeric forms, thereby inducing a shift in the tautomeric equilibrium of the substrate. This could provide an explanation of the fact that the rate of oxidation of I is only about one-tenth of the rate of V.

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24 April 1959

Development of Communication between Young Rhesus Monkeys

Abstract. A communication situation is described in which the rewards of both members of a pair of monkeys cannot exceed chance levels unless the operator monkey responds to cues provided by the informant monkey which indicate the location of food. Performance under these test conditions improved progressively to levels consistently above chance.

Although field studies show that communication is of fundamental importance in the organization and control of nonhuman primate societies, there have been no experimental demonstrations of communication of specific information between monkeys. This report describes an apparatus for the investigation of communication and presents the results obtained in a preliminary experiment (*1*). The test situation, shown schematically in Fig. 1, consisted of two barred restraining cages separated by a table. Four pairs of food carts were mounted on fixed runways on the table, and each pair of carts was connected by an expandable rack so that movement of one cart simultaneously extended the other in the opposite direction. Brass handles were attached to each pair of carts on the operator's side, and all carts were equipped with metal food containers which prevented the operator monkey from seeing the food but permitted the partner (informant) to see it.

Before a trial, both opaque screens and the transparent screen in front of the operator were lowered, the food containers of the appropriate pair of carts were baited, and the one-way vision screen was lowered. The trial was initiated by raising both opaque screens simultaneously and by raising the transparent screen in front of the operator 5 seconds later. (The transparent screen on the informant's side was not used in this experiment.) The operator was permitted only one response per trial (noncorrection procedure), and after this response the operator's choice and the position of the informant prior to the response were recorded. All pairs were tested twice a day, in the morning and the afternoon, and received 24 trials in each test session. The location of the reward varied randomly, with the restriction that each pair of carts was baited with equal frequency in every block of 24 trials.

The subjects were six pairs of rhesus monkeys, approximately 18 months old, born in the laboratory and removed from the mother at birth. All animals had had previous experience in the apparatus in a series of food-sharing tests in which the food was visible to the subjects and in which all responses by the operator were equally rewarded. For communication testing, one member of each pair was arbitrarily designated the operator, and each pair was given a total of 480 training trials (phase 1). At the conclusion of phase 1, the operator and informant roles were reversed and each pair received 1440 trials under the reversed-role condition (phase 2). Upon completion of this phase of the experiment, the subjects were given an additional 480 trials in the original roles (phase 3).

The results for each phase of the experiment are presented in Fig. 2. The data on the informant's position, the only direct measure of informant behavior obtained in the present experiment, are included for purposes of com-