

In this regard it seems to bear some resemblance to the calcium-ammonia reducing system, but differs from it in being far more convenient to handle on a laboratory scale.

Our reagent is also effective in reducing compounds which contain functional groups in the aromatic ring, but a fuller treatment of this subject will be announced later.

CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF PURDUE UNIVERSITY
LAFAYETTE, INDIANA

ROBERT E. ROBINSON
DALE M. SAUVE
OWEN H. THOMAS

RECEIVED NOVEMBER 27, 1953

1,3,5-TRIAZINE^{1,2}

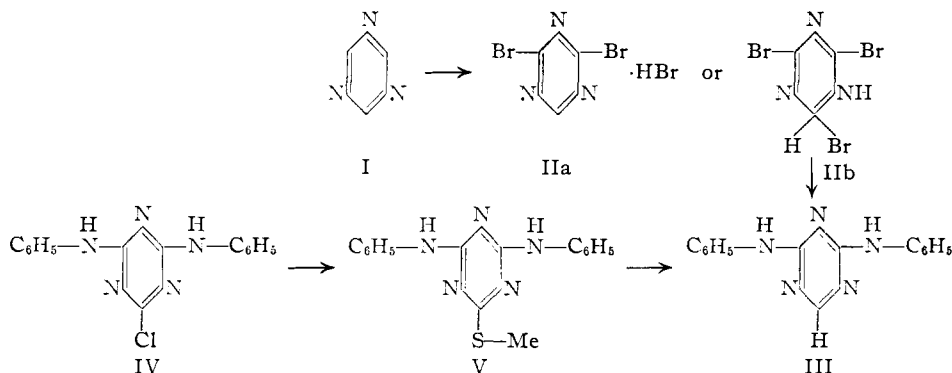
Sir:

Our recently published experiments on the mixed polymerization of nitriles to unsymmetrically substituted triazines with hydrogen halides as catalyst³ failed with the simplest nitrile known, *viz.*, hydrocyanic acid. This led us to a more detailed study of the reaction of hydrocyanic acid with hydrogen chloride the first product of which is the so-called "sesqui-hydrochloride of hydrocyanic acid," $2\text{HCN}\cdot 3\text{HCl}$, recently formulated as $\text{CHCl}_2\text{-NH-CHCl-NH}_2$.⁴ As demonstrated by Hinkel, *et al.*,⁵ this compound easily loses two moles of HCl to a compound $2\text{HCN}\cdot\text{HCl}$, regarded as CHCl=N-CH=NH , which splits off its last molecule of HCl by treatment with dehydrohalogenating agents, *e.g.*, quinoline, to the so-called "dimeric hydrocyanic acid", $\text{C}_2\text{H}_2\text{N}_2$, first obtained by a different route by J. U. Nef.⁶ $\text{C}_2\text{H}_2\text{N}_2$ is consequently then formulated as C=N-CH=NH , iminoformyl-carbonyl-amine.

However, some of the physical as well as chemical properties of this substance raised serious doubts as to its constitution. Therefore we reinvestigated the molecular weight of the "dimeric hydrocyanic acid" which led to the surprising result that this apparently well known substance is really a trimer of hydrocyanic acid, $\text{C}_3\text{H}_3\text{N}_3$.⁷ (Calcd. for $\text{C}_3\text{H}_3\text{N}_3$: mol. wt., 81. Found: mol. wt. (Rast), 89.9, 90.1,

84.0, 88.5; mol. wt. cryoscopic (benzene) 78.0, 78.0.) As hydrolysis even under very mild conditions yields HCOOH and NH_3 quantitatively,⁸ any formula with C-C or N-N groups is definitely excluded. Most obvious is a symmetrical ring structure I suggesting that the "dimeric hydrocyanic acid" is in fact the yet unknown 1,3,5-triazine, the parent compound of so many technical important substances which for a long period many chemists have tried in vain to prepare.

The ultraviolet and the infrared absorption spectra of $\text{C}_3\text{H}_3\text{N}_3$ are closely related to other simple 1,3,5-triazine derivatives of established structure, *e.g.*, 2,4,6-trimethyl-1,3,5-triazine and 2,4,6-trichloro-1,3,5-triazine. The highly symmetrical formula I representing a symmetrically shaped molecule is in agreement with the extreme volatility and the comparatively high melting point (86°) of $\text{C}_3\text{H}_3\text{N}_3$.



Bromination of $\text{C}_3\text{H}_3\text{N}_3$ yields a very sensitive bromo compound which contains three atoms of bromine per one C_3N_3 unit, but is not identical with the known cyanuric bromide. (Calcd. for $\text{C}_3\text{H}_2\text{N}_3\text{-Br}_3$: Br, 74.97. Found: Br, 72.25, 73.18.) This compound reacts with aniline to 2,4-dianilido-1,3,5-triazine (III) (m.p. 316°). Calcd. for $\text{C}_{15}\text{H}_{13}\text{N}_5$: C, 68.40; H, 4.98; N, 26.60. Found: C, 68.36, 68.45; H, 4.95, 5.17; N, 26.60, 26.62) which we have also prepared from 2,4-dianilido-6-chloro-1,3,5-triazine⁹ (IV) through the intermediate V (m.p. $170\text{--}171^\circ$). Calcd. for $\text{C}_{16}\text{H}_{16}\text{N}_6\text{S}$: C, 62.11; H, 4.89; N, 22.64. Found: C, 61.83, 61.80; H, 5.04, 5.05; N, 22.78, 22.89) by a method recently developed for the replacement of halogen by hydrogen in the triazine series.¹⁰ Both compounds were found to be identical which adds a further chemical support to the assumed triazine structure of $\text{C}_3\text{H}_3\text{N}_3$ and also makes it probable that the bromo compound is either 2,4-dibromo-1,3,5-triazine hydrobromide (IIa) or 2,4,6-tribromo-1,2-dihydro-1,3,5-triazine (IIb).

There still remains the task of explaining the formation of triazine from the adducts of hydrocyanic and hydrochloric acid which occurs so easily and under certain conditions with such good yields. Work along these lines is now in progress. A reinvestigation of the known reactions of $\text{C}_3\text{H}_3\text{N}_3$

(1) This article is based on work performed under Project 116-B of the Ohio State University Research Foundation sponsored by the Mathiesen Chemical Corporation, Baltimore, Md.

(2) Triazines VI: communication by Ch. Grundmann, L. Schwenicke and E. Beyer, *Ber.* in press.

(3) Ch. Grundmann, G. Weisse and S. Seide, *Ann.*, **577**, 77 (1952).

(4) L. E. Hinkel and G. H. R. Summers, *J. Chem. Soc.*, 2813 (1952).

(5) L. E. Hinkel, *et al.*, 1834 (1930); 2793 (1932); 674 (1935); 184 (1936); 407 (1940); 1953 (1949); 2813 (1952).

(6) J. U. Nef, *Ann.*, **287**, 377 (1895).

(7) The discoverer of this compound, J. U. Nef,⁶ based the formula $\text{C}_2\text{H}_2\text{N}_2$ on a single cryoscopic determination in benzene, which gave a value of 64 (instead of 54 for $\text{C}_2\text{H}_2\text{N}_2$). Feeling unsatisfied with this result which he attributed to the poor quality of the benzene available in his laboratory, Nef promised a redetermination as soon as he could obtain pure benzene, but apparently this has never been published.

(8) L. E. Hinkel, E. E. Ayling and J. H. Beynon, *J. Chem. Soc.*, 676 (1935).

(9) J. T. Thurston, *et al.*, *THIS JOURNAL*, **73**, 2981 (1951).

(10) Ch. Grundmann, H. Ulrich and A. Kreuzberger, *Ber.*, **86**, 181 (1953).

is also being studied. Some ostensible discrepancies have already been established as experimental errors, but all other reactions and other previously described formations of this compound seem to be compatible with the triazine formula I of $C_3H_3N_3$.

THE OHIO STATE UNIVERSITY
RESEARCH FOUNDATION
COLUMBUS 10, OHIO

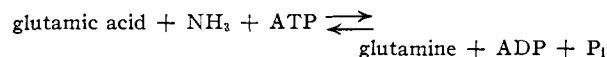
CHRISTOPH GRUNDMANN
ALFRED KREUTZBERGER

RECEIVED DECEMBER, 2, 1953

ON THE MECHANISM OF THE ENZYMATIC SYNTHESIS OF GLUTAMINE¹

Sir:

It has now been established^{2,3} that the synthesis of glutamine by plant enzymes proceeds according to the over-all reaction⁴



The detailed mechanism by which this reaction proceeds, however, has hitherto remained obscure. Data bearing on this mechanism have been obtained by measuring the exchange of phosphate residues between P_i and ATP using the glutamine synthetase obtained from peas and purified 270-fold.²

The absolute amount of such exchange is low as shown in Table I. The way in which the exchange varies with the components of the reaction mixture

TABLE I

PHOSPHATE EXCHANGE BETWEEN RADIOACTIVE INORGANIC ORTHOPHOSPHATE AND ATP CATALYZED BY GLUTAMINE SYNTHETASE

Reaction systems were incubated for 60 min. at 23°. All systems contained: 2.5 μ moles $P^{32}O_4$, 2.0 μ moles ATP, 20 μ moles $MgSO_4$, 10 μ moles cysteine, and 45 μ moles tris-(hydroxymethyl)-aminomethane-HCl (pH 7.4). Additions to this basal mixture as noted above were 50 μ moles glutamate, 40 μ moles NH_3 (or NH_2OH), and 0.5 mg. of enzyme protein. The ATP and P_i were separated chromatographically by the procedure of Bandurski and Axelrod.⁵

Reaction system	C.p.m./ml.	
	ATP	P_i
1 No enzyme	120	900,000
2 Enzyme alone	1140	900,000
3 Enzyme plus glutamate	9800	900,000
4 Enzyme plus glutamate plus NH_3	2600	900,000
5 Enzyme plus glutamate plus NH_2OH	740	900,000

(1) Supported in part by the Polychemicals Department, E. I. du Pont de Nemours and Co., and by a grant-in-aid to J.E.V. from the Charles F. Kettering Foundation.

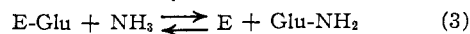
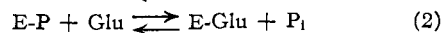
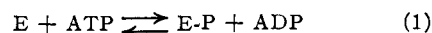
(2) W. H. Elliott, *J. Biol. Chem.*, **201**, 661 (1953).

(3) George C. Webster, *Plant Physiol.*, **28**, 724 (1953).

(4) Abbreviations used: ATP (adenosinetriphosphate), ADP (adenosinediphosphate), P_i (orthophosphate), E (enzyme), Glu (glutamate), Glu- NH_2 (glutamine), CoA (coenzyme A).

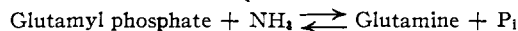
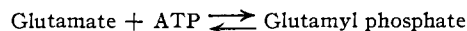
(5) R. S. Bandurski and B. Axelrod, *J. Biol. Chem.*, **193**, 405 (1951).

is however highly significant and in accord with the reaction mechanism



This formulation of reactions accounts for the large increase in exchange which occurs in the presence of glutamate (line 3, Table I) since glutamate is an absolute prerequisite for exchange by this mechanism. The fact that exchange is obtained at all in the presence of only the enzyme, ATP and P_i is probably due to a small amount of ATPase known to be present in the enzyme preparation. The presence of ammonia or hydroxylamine (lines 4 and 5, Table I) results in the formation of glutamine or glutamylhydroxamic acid, thereby decreasing the amount of exchange of P_i by keeping E-Glu at a lower concentration.

The results obtained would seem to preclude the possibility of a glutamyl phosphate acting as an intermediate in glutamine synthesis, since such a mechanism



would require that ammonia be present in order for exchange of P_i into ATP to take place. Similarly, the formation of an adenylyl enzyme as recently implicated⁶ in the synthesis of acetyl-CoA is not consistent with the present results.

The proposed mechanism for the synthesis of glutamine is also consistent with the suggestion of Elliott² that glutamyl transferase activity and glutamine synthetase activity may both reside in a single enzyme. Transferase activity would consist simply of reaction no. 3 above.

Phosphorylation of the enzyme by ATP also has been indicated as the initial step in the synthesis of glutathione from γ -glutamylcysteine and glycine,⁷ and in the synthesis of γ -glutamylcysteine from glutamic acid and cysteine.⁸ The formation of a phosphorylated enzyme, therefore, may represent one general mechanism for the participation of ATP in biological syntheses.

KERCKHOFF LABORATORIES OF BIOLOGY
CALIFORNIA INSTITUTE OF TECHNOLOGY

PASADENA, CALIFORNIA
GEORGE C. WEBSTER
J. E. VARNER

RECEIVED DECEMBER 16, 1953

(6) Mary Ellen Jones, Fritz Lipmann, Helmut Hiltz and Feodor I. y-nen, *THIS JOURNAL*, **75**, 3285 (1953).

(7) John E. Snoke, *ibid.*, **75**, 4872 (1953).

(8) George C. Webster and J. E. Varner, unpublished results.