making his results available to us in advance of publication.

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ADSORPTION ON INORGANIC MATERIALS. I. CATION EXCHANGE PROPERTIES OF ZIRCONIUM PHOSPHATE[1]

Sir:

Precipitates obtained by mixing Zr(IV) and phosphoric acid solutions (to be called zirconium phosphate) were found to exhibit adsorptive properties for cations which apparently can be described in terms of cation exchange behavior. The materials, after precipitation and centrifugation, were dried (or fired), ground to small mesh size and screened. They were then used in small columns of the type commonly used for ion exchange experiments. Distribution coefficients D (amount per kg. adsorber/amount per l. solution) were also determined in batch equilibration experiments involving small amounts of the solids and solutions.

The zirconium phosphates showed excellent adsorptive properties for a number of cations, *e.g.*, the alkali metals, alkaline earths, Al(III), Fe(III), etc., and typical cation exchange displacement reactions. They appear to have reasonable adsorptive capacities. For example, zirconium phosphate dried at 25° can adsorb *ca.* 1 mole Cs^+ from 0.1 M CsCl solutions. This uptake decreases with firing temperature, though not seriously if the firing temperature does not exceed 200°, where uptake is still 0.7 mole of Cs^+ per kg. Although detailed rate studies have not been carried out as yet, columns prepared from these materials seem to behave qualitatively similarly to columns prepared with conventional organic cation exchange resins.

The exchange reaction appears to approach ideal behavior, at least under trace conditions (low loading). Thus log D for tracer Ce(III) is a linear function of log M HCl with slope *ca.* minus three, as expected for ideal Ce^{+++}–H^+ exchange.

Detailed studies of the selectivities have so far only been carried out for the alkali metals. For these the selectivities differ widely, permitting separations with small columns. A typical separation of Rb and Cs with a small column of room temperature dried zirconium phosphate is illustrated in Fig. 1.

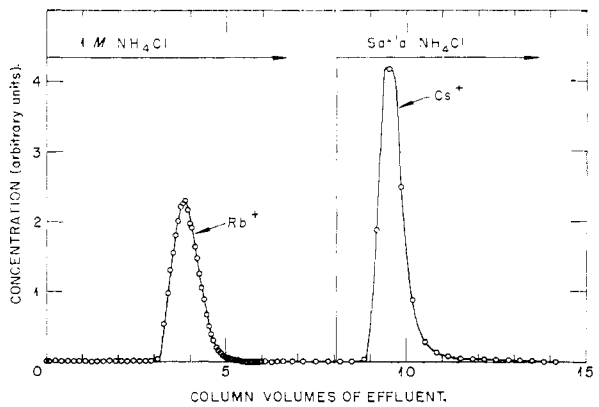

Fig. 1.—Separation of Rb^+ and Cs^+ on zirconium phosphate (0.25 cm.^{2} × 12 cm. column) (flow rate 0.8 cm./min.).

(1) This document is based on work performed for the U. S. Atomic Energy Commission at the Oak Ridge National Laboratory.

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A TETRAHYDRO-FOLIC ACID LINKED FORMIMINO TRANSFER ENZYME

Sir:

We wish to report the occurrence of a formimino (–CH=NH) transferring enzyme for formiminoglycine in extracts of *Clostridium acidi-urici* and the function of tetrahydro-folic acid in formimino transfer.

Formiminoglycine (FIG), identified by Rabinowitz and Pricer[1] as an intermediate in purine degradation by *Clostridium cylindrosporum*, has been prepared by the method of Micheel and Flitsch.[2] *Clostridium acidi-urici* cells, grown in a uric acid medium essentially as outlined by Barker and Beck,[3] were ruptured as a frozen cell paste by Hughes press.[4] After centrifugation, the "crude" extract was used or "Dowex" treated (15 minutes at 0° with 3 g. Dowex-1-Chloride/800 mg. bacterial protein) to remove cofactors.

The "crude" extract and the "Dowexed" extract when supplemented with tetrahydro-folic acid (THFA), cleave formiminoglycine as shown

$$\text{Formiminoglycine} \xrightarrow{\text{THFA}} \text{glycine} + \text{ammonia} + \text{formate} \quad (1)$$

Formiminoglycine was measured by the alkaline nitroprusside–ferricyanide method of Rabinowitz and Pricer[5] and glycine, after elution from chromatographs, by the ninhydrin method of Moore and Stein.[6] Separation and qualitative identification were achieved by chromatography on Whatman no. 1 paper with a phenol-water solvent. Formiminoglycine (R_f 0.75) and glycine (R_f 0.38) were visualized by using their respective colorimetric reagents as sprays.[7] Formiminoglycine was stable to incubation and chromatography as well as to the procedure for ammonia analysis.[8] As observed in Table I, the "crude" extract decomposed formiminoglycine beyond the glycine stage—*i.e.*, recovery of but 0.5 μM. glycine and 1.4 μM. ammonia/μM. formiminoglycine used. "Dowexed" extract did not cleave formiminoglycine but was activated by tetrahydro folic acid to give essentially

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(7) H. K. Berry, H. E. Sutton, L. Cain and J. S. Berry, Univ. Texas Pub. No. 5109 (1951).
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a stoichiometric yield of glycine and ammonia. N-Formylglycine was inactive and thus not considered as an intermediate in the conversion of formiminoglycine to glycine.

TABLE I

FUNCTION OF TETRAHYDROFOLIC ACID IN FORMIMINOGLYCINE DEGRADATION

Thunberg tube, 4.5 ml.; 100 μM. phosphate buffer, *p*H 7.5; 3 μM. $Na_2S_2O_4$; extract (4 mg. bacterial nitrogen); 1 mg. THFA where indicated (prepared according to Broquist, THIS JOURNAL, **73**, 3535 (1951) half life in absence of O_2 about 2 weeks); after evacuation, 40 μM. substrate tipped from side arm; incubated 60′ at 35°.

Substrate and enzyme	Substrate used, μM.	Products formed Glycine	NH_3 μM.
Formiminoglycine, 40 μM.			
Extract, crude	40	21	56
Extract, Dowex	0	0	0
Extract, Dowex + THFA, 1 mg.	38	36	42
N-Formylglycine, 40 μM.			
Extract, crude	0	0	..
Extract, Dowex + THFA, 1 mg.	0	0	..

These observations suggested to us that formiminotetrahydrofolic acid might occur as an intermediate as

$$\text{Formiminoglycine} + \text{THFA} \rightleftharpoons \text{glycine} + \text{formimino-THFA} \quad (2)$$

$$\text{Formimino-THFA} \xrightarrow{H_2O} \text{THFA} + \text{ammonia} + \text{formate} \quad (3)$$

To test this postulate, 40 μM. glycine-2-C^{14} and 40 μM. unlabeled formiminoglycine were incubated with "Dowexed" extract, alone and with added tetrahydro-folic acid. Aliquots were chromatographed as described above and the chromatographs scanned for radioactivity with an actigraph (Nuclear instrument, Model C-100) previously calibrated with glycine-2-C^{14} of known activity. As shown in Table II, the radioactivity of glycine-2-C^{14} was equilibrated rapidly with formiminoglycine in a tetrahydrofolic acid and enzyme dependent reaction. Formylglycine does not exchange with glycine-2-C^{14} nor form formiminoglycine. Hydrolysis of the formiminoglycine formed yielded C^{14} in the glycine only. The reaction sequence beyond "formimino-tetrahydrofolic acid," of which one route leads to ammonia plus formate (reaction 3), is slower than the exchange. Preliminary data indicate that the formimino carbon from formiminoglycine (C_8 of purine) may become available for hydroxymethylation,[9] thus implying a variety of reactions and enzymatic steps beyond the postulated "formimino-tetrahydrofolic acid" intermediate. The activity of tetrahydrofolic acid with these unfractionated extracts does not impinge upon the coenzyme form of this factor.[10]

TABLE II

FORMIMINO TRANSFER TO GLYCINE-2-C^{14}; TETRAHYDROFOLIC ACID DEPENDENCE

Protocol as Table I; substrates, 40 μM. glycine-2-C^{14} (SA 3150 dis./sec./μM.); 40 μM. formiminoglycine; 1 mg. THFA where added.

Incubation, min.	C^{14}, dis./sec./μM. Glycine	Formimino-glycine
0	3150	0
2	2210	905
5	1650	1230
15	1530	1310
15, w/o extract	3150	0
15, w/o THFA	3150	0

The accumulation of formiminoglutamic acid from histidine in folic acid deficient rats,[11,12] and in preparations of mammalian liver[13] and of microorganisms,[14] suggests a similar sequence for this substrate and thus that formimino transfer may constitute a biologically important reaction type.

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THE STEREOCHEMISTRY OF BASE-CATALYZED ADDITIONS OF THIOLS TO ACETYLENES

Sir:

Although a high degree of stereospecificity has been realized for the addition of hydrogen, halogens and hydrogen halides to acetylenes, similar studies on *nucleophilic* additions have not been published.[1] We have now observed that base-catalyzed additions of thiols to the acetylenic compounds, phenylacetylene, 2-butyne, chloroacetylene and *p*-tolylmercaptoacetylene, proceed in a *trans* fashion.

Refluxing an alcoholic solution of phenylacetylene with sodium *p*-toluenethiolate for 15 hours resulted in a 79% yield of *cis*-ω-styryl *p*-tolyl sulfide (none of the *trans* isomer was isolated), which was readily oxidized by hydrogen peroxide to its sulfone, m.p. 76–77°, λ_{max} 266 mμ, ϵ_{max} 14 × 10^3; *trans* form,[2] m.p. 121°, λ_{max} 276 mμ, ϵ_{max} 25.9 × 10^3. *trans*-ω-Styryl *p*-tolyl sulfone also has been prepared by the Friedel–Crafts reaction of *trans*-ω-styrenesulfonyl chloride[3] with toluene.[4]

Similar results were obtained with sodium methanethiolate and phenylacetylene, the product being methyl *cis*-ω-styryl sulfide, yield 73%, b.p. 101.5° (5 mm.). (*Anal.* Calcd. for $C_9H_{10}S$: C, 71.95; H, 6.71. Found: C, 71.28; H, 6.88.) Its sulfone, m.p. 66–67°, λ_{max} 261 mμ, ϵ_{max} 19.9 × 10^3, was different from the *trans* isomer[4], m.p. 78–79°, λ_{max} 264 mμ, ϵ_{max} 23.7 × 10^3. The 78–79°

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