Shemin, *et al.*,[3] have postulated how a reduced tetrapyrrole of the type III configuration might be formed by the coupling of two dipyrroles arising from porphobilinogen. Confirmatory evidence is noted in the work of Bogorad[4] and Granick,[5] who have found colorless intermediates between porphobilinogen and protoporphyrin which undergo autoxidation to uroporphyrin and coproporphyrin.

We have isolated uroporphyrin III from Turacus feathers according to Nicholas and Rimington.[6] The uroporphyrin was then reduced with sodium amalgam by a modification of Fischer's method.[7] This reduction results in the addition of hydrogen atoms to the four methene bridge carbon atoms and two of the pyrrole nitrogen atoms. The colorless non-fluorescent product reoxidized in air to uroporphyrin, which was identified by its absorption spectrum.

This reduced uroporphyrin III (RUP) in a lysed duck erythrocyte system[8] was found after incubation to increase the levels of free uroporphyrin, coproporphyrin, and protoporphyrin. Under similar conditions but with added Fe^{59}, RUP also significantly increased the radioactivity of the hemin,[9] indicating increased biosynthesis (Table I). Lowered Fe^{59} uptake found with the higher concentrations of RUP may be due to an inhibitor resulting either from a side reaction in the reduction process,[10] or from an impurity accompanying the uroporphyrin during its isolation.[6] Inhibition due to the RUP preparation was never seen below 0.30 μ mole. No increased labelling of the hemin with Fe^{59} was noted in samples incubated with uroporphyrins I or III or with reduced uroporphyrin I.

TABLE I

Fe^{59} INCORPORATION INTO HEME WITH ADDED RUP

Experiment	RUP added (μmole)	CPM (hemin)[9]
I	0	1680
	0.044	2580
	.088	4120
	.176	8720
II		1870
	.10	6000
	.20	7100
	.30	18000
	.40	7500
	.60	4730
	.80	1950
	1.00	1080

Experimental conditions: washed duck erythrocytes lysed with 1.5 vol. dist. H_2O,[8] made isotonic with sucrose, and 20 ml. used per sample. Incubation 9 hr. at room temp. with shaking; 1 μc Fe^{59} added per sample. Radioactivity of hemin in KOH solution determined with scintillation well counter.

(3) D. Shemin, C. S. Russel and T. Abramsky, *J. Biol. Chem.*, **215**, 613 (1955).

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In order to determine whether RUP is a direct intermediate in heme biosynthesis, an isotope dilution experiment was carried out using glycine-2-C^{14}, a known precursor of heme. Data in Table II show the markedly reduced glycine incorporation in the presence of RUP, a finding of particular importance in view of the small amount added. Since inhibition has never been noted at these substrate levels, the RUP appears to have diluted the labelled glycine and therefore to be in the main pathway of heme biosynthesis. Preliminary data indicate that reduced coproporphyrin III similarly serves as a heme precursor, but less effectively than RUP.

TABLE II

GLYCINE-2-C^{14} DILUTION BY RUP

RUP added	CPM (hemin)
None	82
0.15 μmole	50
.22 μmole	40
.29 μmole	37

27 μmole (1.3 μc.) glycine-2-C^{14} per sample. Experimental conditions: Table I. C^{14} activity determined with end window counter; values corrected to infinite thickness of hemin.

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RECEIVED NOVEMBER 28, 1955

XYLULOSE 5-PHOSPHATE AND THE FORMATION OF SEDOHEPTULOSE 7-PHOSPHATE WITH LIVER TRANSKETOLASE

Sir:

Mitsuhashi and Lampen,[1] Hochster,[2] and Slein[3] have obtained evidence for a phosphate ester of D-xylulose as an intermediate in the metabolism of D-xylose in bacterial extracts. The first isolation of xylulose phosphate was that of Ashwell and Hickman,[4] who found this product to be formed when ribose 5-phosphate was incubated with spleen extracts and obtained evidence for a new enzyme which was responsible for its appearance.

The formation of xylulose phosphate now can be represented by the equation

Ribulose 5-phosphate (Ru-5-P) $\rightleftharpoons$ xylulose 5-phosphate (Xu-5-P)

This enzyme occurs in extracts of *Lactobacillus pentosus*,[5] muscle,[6,7] spleen,[8] and spinach, and has been named phosphoketopentoepimerase (epimerase). Epimerase has been purified more than 100-fold from extracts of *L. pentosus* by a procedure involving manganous salt precipitation, ammonium sulfate fractionation, selective heat denaturation, and calcium phosphate gel adsorption. The

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(5) P. K. Stumpf and B. L. Horecker, *J. Biol. Chem.*, in press.

(6) F. Dickens and D. H. Williamson, *Nature*, **176**, 400 (1955).

(7) P. A. Srere, J. Cooper, V. Klybas and E. Racker, *Arch. Biochem. Biophys.*, **59**, 535 (1955).

(8) G. Ashwell and J. Hickman, unpublished work.

purified preparations are free of phosphoriboisomerase and transketolase activity.

Xu-5-P has been isolated by Stumpf and Horecker[5]; with epimerase purified from *L. pentosus* an improved procedure has been developed. In the presence of this enzyme and spinach phosphoriboisomerase,[9] ribose 5-phosphate (R-5-P) is converted to an equilibrium mixture containing (at 38°) about 50% of the total pentose esters as Xu-5-P, measured with xylulose kinase[5] following enzymatic hydrolysis. The remaining R-5-P and Ru-5-P can be converted with ATP and phosphoribulokinase[9] to ribulose diphosphate which is separated from Xu-5-P by fractional precipitation of the barium salts.

TABLE I

EPIMERASE REQUIREMENT WITH LIVER TRANSKETOLASE

Reaction	Triose phosphate[a]
Complete system[b]	9.8×10^{-3}
Epimerase omitted	1.2×10^{-3}

[a] Micromoles formed per minute. [b] The complete system contained 18 micrograms of bacterial epimerase and an excess (34 micrograms) of purified liver transketolase[10] rich in phosphoriboisomerase; the substrate was R-5-P. The test system previously described[10] was employed except that the glycerophosphate dehydrogenase preparation contained very little aldolase.

TABLE II

TRANSKETOLASE ACTIVITY WITH VARIOUS PENTOSE PHOSPHATE ESTERS

Addition	Experiment 1,[a] triose phosphate formation, micromoles/min. $\times 10^{-3}$	Experiment 2,[b] S-7-P formation, micromoles/10 min.
R-5-P	0	0.04
Xu-5-P	0.2	.06
R-5-P + Xu-5-P	1.9	.46
Ru-5-P	0.4	.15
R-5-P + Ru-5-P	0.3	.08

[a] In this experiment triose phosphate formation was measured in the spectrophotometric test of Warburg and Christian,[11] except that glycylglycine buffer *p*H 7.4 was used instead of pyrophosphate. The substrate concentration was 10^{-4} *M*; the total volume was 1.0. 15 Micrograms of transketolase was added. [b] In this experiment the reaction mixture (0.80 ml.) contained 510 micrograms of liver transketolase, 1.4 μmoles of pentose phosphate (total) and 5 μmoles of cysteine in 0.01 *M* glycylglycine buffer, *p*H 7.4. S-7-P was determined by means of the Dische diphenylamine test.[12]

With the aid of purified epimerase and Xu-5-P, it can be shown that the substrate for liver transketolase, as claimed for the yeast enzyme,[7] is Xu-5-P, rather than Ru-5-P. Thus, the liver preparations will form triose phosphate from R-5-P or Ru-5-P at a slow rate, unless the purified epimerase is added (Table I). The requirement for epimerase is absent when a mixture of Xu-5-P and R-5-P is used as substrate (Table II). Neither ester alone is active and Ru-5-P, alone or in combination with R-5-P, shows little activity. These results are consistent with the hypothesis[13] that ribose 5-phosphate acts as an acceptor for "active glycolaldehyde." A similar requirement for epimerase has been demonstrated with highly purified spinach transketolase.[14]

The requirement for epimerase explains the previously reported effect of aldolase in the formation of sedoheptulose 7-phosphate (S-7-P) from R-5-P.[10] This can now be related to the capacity of muscle aldolase preparations to catalyze the conversion of Ru-5-P to Xu-5-P. Recrystallized preparations were analyzed for epimerase, which was found to be present in each case in amounts approximately proportional to the aldolase activity (Table III).

TABLE III

THE FORMATION OF Xu-5-P FROM Ru-5-P WITH CRYSTALLINE MUSCLE ALDOLASE

Preparation[a]	Aldolase activity (*A*),[b] μmoles/min.	Epimerase activity (*E*),[c] μmoles/min.	*E/A*
1	28.0	0.30	0.011
2	12.0	.16	.013
3	18.1	.27	.015
4	40.7	.56	.014

[a] Recrystallized from 2 to 5 times.[15] Preparation 3 had been stored at 2° for 3.5 years. Preparation 4, obtained by a modification of the procedure of Beisenherz, *et al.*,[16] was kindly provided by Dr. B. Bloom of this Institute. [b] The spectrophotometric assay procedure of Warburg and Christian[11] was used, with crystalline muscle glyceraldehyde 3-phosphate dehydrogenase.[17] [c] Epimerase activity was assayed in the transketolase test system.[10] Rat liver transketolase was present in excess (0.10 unit). The substrate was R-5-P (0.24 μmole).

The presence of epimerase activity in recrystallized aldolase is remarkable, since these preparations, unlike myogen A,[18] are free of triose phosphate isomerase and glycerophosphate dehydrogenase. However, Racker and his co-workers[19] have obtained crystalline aldolase preparations free of epimerase, and this activity must therefore be regarded as a contaminant. This is confirmed by the results of heat and acid inactivation experiments in which it was possible to reduce aldolase activity by more than 90%, with only 40 to 50% loss of epimerase activity. The purified bacterial epimerase preparations are free of aldolase.

It is thus evident that transketolase from liver or spinach, like the yeast enzyme,[7] is virtually inactive with Ru-5-P. All of the substrates for this enzyme, including D-xylulose 5-phosphate,[5] sedoheptulose 7-phosphate,[10] D-fructose 6-phosphate,[20] and L-erythrulose,[10] can assume the same configuration on carbon atoms 1 through 4. It will be of interest to examine the reactivity of transketolase with L-ribulose 5-phosphate and L-xylulose 5-phosphate, when these substances become available.

We are indebted to Dr. E. Racker for kindly

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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.

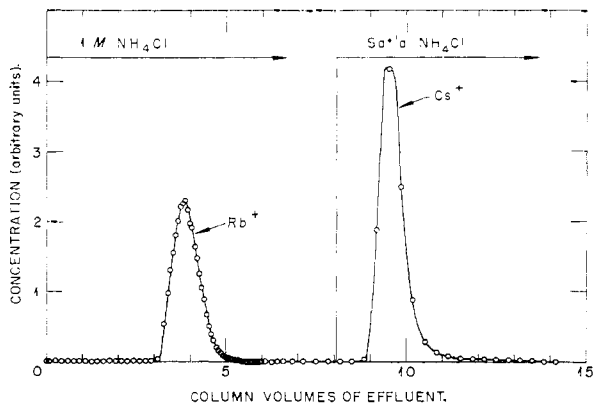

Fig. 1.—Separation of Rb^+ and Cs^+ on zirconium phosphate (0.25 cm.^{2} × 12 cm. column) (flow rate 0.8 cm./min.).

(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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