cytochrome *c* reductase, prepared from guinea pig by a method similar to that of Edelhoch, *et al.*,[2] with this hydroxylamine inhibiting factor, promotes an exchange reaction between P_{32} and ATP,[3] in a system containing ADP, DPNH and Mg^{++}. This exchange does not require cytochrome *c* or oxygen, and DPNH can be substituted by DPN and, furthermore is not inhibited by 2–4 dinitrophenol or fluoride. As this exchange depends on the presence of reductase and DPN or DPNH we are dealing with oxidative phosphorylation.

Experiments were performed with a digitonin extract, prepared by extracting rat liver mitochondria with 2% digitonin in 0.55 *M* KCl–0.1 *M* TRIS *p*H 7.5–0.1 *M* reduced glutathione. This extract together with cytochrome *c* reductase and hydroxylamine inhibiting factor was tested for oxidative phosphorylation in a system containing: (a) alcohol and alcohol dehydrogenase as electron source, (b) cytochrome *c* as electron acceptor, (c) glucose plus hexokinase to trap the ATP formed, (d) ITP which is necessary in this system. Glucose 6-phosphate is not metabolized in this system, and was determined by the increase of absorption at 340 mμ with TPN and glucose 6-phosphate dehydrogenase.[4]

TABLE I

TRIS buffer *p*H 7.5, 80 μM.; ADP 2.4 μM; KCl 36 μM.; $MgCl_2$ 4 μM.; $MnCl_2$ 1 μM.; glucose 6 μM.; ITP 0.3 μM; cytochrome *c* 0.33 μM.; DPN 1 μM; ethanol 200 μM.; orthophosphate 5 μM.; alcohol dehydrogenase 220 units; hexokinase 70 units; cytochrome *c* reductase approx. 1 mg.; hydroxylamine inhibiting factor approx. 1 mg.; mitochondria extract approx. 3 mg.; total volume 0.9 ml., incubated during 15 min., at 20°, under nitrogen.

	μM., glucose 6-phosphate
Complete	0.400
Without cytochrome *c* and DPN	0.015
Without mitochondria extract	0.115
Without hydroxylamine inhibiting factor	0.057
Without mitochondria extract and reductase	0.115
Complete	0.576
Without ITP	0.144
With $10^{-4}M$ dinitrophenol	0.150
With $10^{-4}M$ 8-hydroxyquinoline	0.132
Complete	0.298
With 0.04 *M* hydroxylamine	0.087
Without ITP	0.168
Complete, with 0.03 *M* fluoride	0.360
Same, with 10^{-4} *M* dinitrophenol	0.194

The present data show that phosphorylation depends on cytochrome *c* and ITP, and is inhibited as *in situ* by 2,4-dinitrophenol. Inhibition by 8-hydroxyquinoline and hydroxylamine relates our system to a metallo-flavoprotein.[5]

Although no measurements were made on cytochrome *c* reduction, present data suggest an ATP/-cytochrome *c* reduction ratio with a value above 1. The present enzymes seem to be responsible for two of the three phosphorylations occurring during DPNH oxidation in mitochondria.

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(2) H. Edelhoch, O. Hayashi and L. O. Teply, *J. Biol. Chem.*, **197**, 97 (1952).

(3) ADP = adenosine diphosphate, ATP = adenosine triphosphate, DPN = diphosphopyridine nucleotide, DPNH = reduced diphosphopyridine nucleotide, ITP = inosine triphosphate, TRIS = tris (hydroxymethyl) aminomethane.

(4) A. Kornberg, *J. Biol. Chem.*, **167**, 805 (1950).

(5) D. E. Green, *Science*, **120**, 7 (1954).

THE PARTIAL CHARACTERIZATION OF A HISTAMINE METABOLITE FROM RAT AND MOUSE URINE

Sir:

Considerable work has been done during the past few years on the isolation and characterization of metabolites from rat and mouse urine after the injection of C^{14}-labeled histamine.[1]

The urine of rats which had been injected by Schayer with large amounts of low-isotopic histamine labeled with C^{14} in the 2-position of the imidazole ring was processed and several radioactive metabolites were isolated. One of the metabolites, isolated through the use of Dowex 50 columns with ammonium formate buffer, was crystalline in ethanol–ether mixtures, but on exposure to air it reverted to an oil. The hydrochloride was crystalline, m.p. 166–167°, $[\alpha]^{25}D$ −49.4° (methanol, c = 0.7%). Calcd. for $C_{10}H_{14}N_2O_6 \cdot HCl$: C, 40.75; H, 5.13; N, 9.51; Cl, 12.03. Found: C, 41.21; H. 5.46; N, 9.30; Cl, 11.99.

The analysis and hydrolytic data suggested a pentose-imidazoleacetic acid conjugate similar to that mentioned recently in unpublished work by Tabor.[2]

The conjugate is not hydrolyzed on heating at 100° for five hours in 12 *N* hydrochloric acid. It is readily hydrolyzed, however, in five hours in sealed tubes at 145° with 0.1 *N* acid. The hydrolyzed metabolite solution is applied directly to paper chromatograms.

Imidazoleacetic acid was shown to be present by comparative runs against a known sample, the developing spray being the Pauly reagent.[3]

In pyridine 65, water 35, the known and the hydrolysis solution gave spots with R_F values of 0.77 and 0.79, resp. In *n*-butanol 8, ethanol 2, water 2, both spots had an R_F of 0.18, and in *n*-butanol 4, ethanol 1,1, water 1.9 they both had an R_F of 0.39.

The sugar component was determined by spraying the paper chromatograms with 1% aniline oxalate in glacial acetic acid.[4]

The sugar was found to be ribose by R_F comparisons in three solvent mixtures: *n*-butanol 3, pyridine 2, water, 1.5 (R_F 0.57); ethyl acetate 2, pyridine 1, water 2 (upper phase), (R_F 0.49); and collidine-water (R_F 0.67).

The course of hydrolysis was followed by noting the increase in formation of imidazoleacetic acid and ribose on paper chromatograms, and by a decrease in concentration of the metabolite, the

(1) (a) R. W. Schayer, *J. Biol. Chem.*, **196**, 469 (1952); (b) A. H. Mehler, H. Tabor and H. Bauer, *ibid.*, **197**, 475 (1952); (c) H. Tabor, A. H. Mehler and R. W. Schayer, *ibid.*, **200**, 605 (1953); (d) L. P. Bouthillier and M. Goldner, *Arch. Biochem. Biophys.*, **44**, 251 (1953).

(2) H. Tabor, *Pharmacol. Rev.*, **6**, 299 (1954) (unpublished data of O. Hayaishi and H. Tabor).

(3) G. Hunter, *Biochem. J.*, **22**, 4 (1928).

(4) L. Hough, J. K. N. Jones and W. H. Wadman, *J. Chem. Soc.*, 1702 (1950).

latter having an R_F of 0.19 in *n*-butanol 4, ethanol 1.1, water 1.9. The spot was developed by the periodate method.[5]

This metabolite from rat urine must then be 1-(3)-ribosyl-imidazole-4-(5)-acetic acid. A similar metabolite which gave the same components on hydrolysis was isolated from mouse urine. It melted at 170–173°, the difference in melting points probably being due to a small difference in purity between the two samples, since a mixed melting point was not depressed.

As far as is known, this is the first instance in which ribose appears to participate in a detoxication mechanism. No reference to sugars other than glucuronic acid is made, for example, by Williams.[6]

Further work is being carried out on the characterization of the ribose ring structure, and will be published in detail elsewhere at a later date.

The author is indebted to B. W. Turnquest III for carrying out the chromatographic isolation of the metabolite and to R. W. Schayer for supplying the large quantities of urine required and for aid in measuring the radioactivity of the chromatographic samples.

(5) R. L. Metzenberg and H. K. Mitchell, THIS JOURNAL, **76**, 4187 (1954).

(6) R. T. Williams, "Detoxication Mechanisms," John Wiley and Sons, Inc., New York, N. Y., 1947.

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THE EXCRETION OF IMIDAZOLEACETIC ACID RIBOSIDE FOLLOWING THE ADMINISTRATION OF IMIDAZOLEACETIC ACID OR HISTAMINE TO RATS[1]

Sir:

In previous reports a number of chromatographically separate products have been described in the urine following the administration of C^{14}-histamine.[2a,2b] Imidazoleacetic acid (ImAA) has been identified as one of these products in the rat.[2b,3a,3b] In this communication we are reporting the *in vivo* conversion in the rat of ImAA to ImAA-riboside.

9.3 millimoles of ImAA[4] ($C^{14}OOH$ labeled; specific activity: 106 c.p.m./μM.) was administered intraperitoneally to a total of 30 rats (av. wt. 200 g.) in 4 divided doses (at two-hour intervals). The urine was collected for 18 hr.,[5] adsorbed on Dowex 50 (height 33 cm., diameter 3.2 cm.), and eluted with HCl (gradient elution 500 ml. $H_2O \rightarrow 2$ *N* HCl; volume of fractions 25 ml.). Two radioactive peaks were observed. The material in peak 1 (fractions 28–34) contained 55% of the counts, and did not couple with diazotized *p*-nitroaniline. Peak 2 (fractions 42–55) contained 45% of the counts, and appeared to contain unchanged imidazoleacetic acid as demonstrated by paper chromatography[6] and paper electrophoresis.[7] Peak 1 was then chromatographed on Dowex-1-acetate, rechromatographed on Dowex 50, and crystallized from acetone–water. *Analysis*[8]*:* Calculated for $C_{10}H_{15}O_6N_2Cl$: C, 40.75; H, 5.13; N, 9.51; Cl, 12.03. Found: C, 40.55; H, 5.36; N, 9.35; Cl, 12.21. *Specific Activity* 110 c.p.m./μM. *Melting point:* 172–176.[9] *Titration: pK* 3 and 6.1. The compound reacted with 1 equivalent of periodate. The isolated compound was hydrolyzed[10] to ImAA and ribose by a partially purified nucleosidase from *Lactobacillus delbruckii*.[11] The ribose was identified by paper chromatography,[12] and by identification of the tetrabenzoate derivative as β-D-ribopyranose tetrabenzoate.[13] The ImAA was purified by Dowex chromatography, crystallized from acetone–water, and sublimed (m.p. 214–216°); it was further identified by paper chromatography,[6] paper electrophoresis,[7] by comparison of the infrared spectrum with that of authentic ImAA,[14] and by assay with Pseudomonas ImAA reductase.[15] The structure of the isolated compound is tentatively identified as 1(3)-D-ribofuran-

(1) Accounts of this work have been presented at the fall meeting of the American Society for Pharmacology and Experimental Therapeutics at Charlottesville, Va., Sept. 8, 1954, and at the Amino-Acid Symposium held at the McCollum-Pratt Institute, The Johns Hopkins University, June, 1954.

(2) (a) R. W. Schayer, *J. Biol. Chem.*, **196**, 469 (1952); (b) L. P. Bouthillier and M. Goldner, *Arch. Biochem.*, **44**, 251 (1953).

(3) (a) A. H. Mehler, H. Tabor and H. Bauer, *J. Biol. Chem.*, **197**, 475 (1952); (b) H. Tabor, A. H. Mehler and R. W. Schayer, *ibid.*, **200**, 605 (1953).

(4) Imidazoleacetic acid ($C^{14}OOH$) was prepared by alkaline hydrolysis of imidazoleacetonitrile; the latter was obtained by treating histidine-α-C^{14} with sodium hypochlorite (H. Bauer and H. Tabor, unpublished method).

(5) Essentially all of the isotope was recovered in the urine; this is in agreement with the findings of (5a) L. P. Bouthillier and G. Léveillé, THIS JOURNAL, **75**, 4075 (1953). Essentially no $C^{14}O_2$ (<1%) was found in the respiratory CO_2.

(6) The R_F of peak 2 and authentic ImAA were identical in propanol 75, 1 *N* acetic acid 25[6a] (0.34); propanol 75, 1 *N* NH_4OH 25[6a] (0.38), *t*-butanol 70, formic acid 15, H_2O 15[6b] (0.58), and *n*-butanol 80, ethanol 10, concd. NH_4OH 30[2a] (0.08). The corresponding R_F values obtained for peak 1 were 0.21, 0.28, 0.42, and 0.03. (b) A. Meister, H. Sober and S. Tice, *J. Biol. Chem.*, **189**, 577 (1951); (a) B. N. Ames and H. K. Mitchell, THIS JOURNAL, **74**, 252 (1952).

(7) Good separation of the two components was obtained by paper electrophoresis in 0.025 *M* potassium phosphate buffer (*p*H 6.5); 900 volts, 1.5 hr., Whatman No. 1 paper. Essentially no migration was observed with peak 2 material or with authentic ImAA. Peak 1 material migrated 8 cm. toward the positive pole.

(8) The analyses were carried out by Dr. William Alford of the Microanalytical Laboratory of this Institute.

(9) Kofler block; the melting point varied considerably with the speed of heating, and with slow heating considerably lower melting points (*e.g.*, 159–163° with decomposition) were observed.

(10) Essentially no hydrolysis was observed in 2 *N* HCl at 100° overnight. Complete hydrolysis was carried out in concentrated HCl in a sealed tube for 48 hr. at 150° (J. M. Gulland and T. F. Macrae, *J. Chem. Soc.*, 662 (1933)). The ribose was carbonized but the free ImAA was isolated and characterized.

(11) Extracts of *L. delbruckii* hydrolyze β-ribosidic linkages (H. M. Kalckar, personal communication). Partial purification was effected by an ammonium sulfate fractionation (40–55%). The hydrolysis mixture containing 0.5 mM. of ImAA riboside, 2 mM. potassium phosphate (*p*H 6.2), and enzyme (50 mg. protein) in 100 ml. volume, was incubated at 25° for 3 hr. The reaction was followed by the appearance of material giving the orcinol and Pauly reactions.

(12) The hydrolysis product and authentic ribose had the same R_F in 90% isopropanol and 77% acetone (R_F 0.43 and 0.61, respectively).

(13) β-D-Ribose tetrabenzoate was prepared by a modification of the method of R. Jeanloz, H. G. Fletcher, Jr., and C. S. Hudson, THIS JOURNAL, **70**, 4052 (1948). We wish to thank Dr. H. G. Fletcher, Jr., for his advice in this preparation, as well as for supplying various samples of authentic pentose tetrabenzoates. The melting point of the tetrabenzoate of the hydrolysis product was 129–130.5°, of authentic β-D-ribose tetrabenzoate 129.5–131.5° (lit. 131°); mixed 129–131.5°. The infrared spectra[14] of the tetrabenzoate of the hydrolysis product in $CHCl_3$ were identical with the authentic material, and different from those observed with several other pentose tetrabenzoates.

(14) We wish to thank Mr. H. K. Miller for the various infrared spectra.

(15) O. Hayaishi, H. Tabor and T. Hayaishi, THIS JOURNAL, **76**, 5570 (1954).