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.

RHEUMATIC FEVER RESEARCH INSTITUTE
NORTHWESTERN UNIVERSITY S. A. KARJALA
CHICAGO 8, ILLINOIS

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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).

osyl imidazoleacetic acid hydrochloride, although the exact site of substitution on the imidazole ring remains to be determined.

We have also isolated ImAA-riboside as a major product (*ca.* 60%) in the urine after the administration of histamine (α-C^{14}) intraperitoneally to rats (43 micromoles per 250 g. rat). These results agree with those of Karjala,[16] and may represent one of the radioactive peaks reported by Schayer[2a] and by Bouthillier and Goldner.[2b]

This isolation of ImAA-riboside from rat urine extends our previous report[3a,3b] that ImAA is a major intermediate in the metabolism of histamine, and demonstrates its further conversion to ImAA-riboside.[17] In view of the importance of other ribosides in cellular structure and metabolism, the formation of ImAA riboside may reflect the existence of a cellular mechanism for the synthesis of ribosides of other compounds containing the imidazole structure.

(16) S. A. Karjala, THIS JOURNAL, in press.

(17) The percentage of administered ImAA recovered as free ImAA increases as the quantity of ImAA administered increases. This may account for the report of Bouthillier and Léveillé (Ref. 5a) that no further metabolic products of ImAA could be demonstrated in the rat.

NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH, BETHESDA 14, MARYLAND AND DEPARTMENT OF MICROBIOLOGY WASHINGTON UNIVERSITY SCHOOL OF MEDICINE ST. LOUIS, MISSOURI

HERBERT TABOR
OSAMU HAYAISHI

RECEIVED NOVEMBER 12, 1954

THE ENZYMATIC FORMATION OF PORPHOBILINOGEN FROM δ-AMINOLEVULINIC ACID AND ITS CONVERSION TO PROTOPORPHYRIN[1]

Sir:

We have previously demonstrated that not only is δ-aminolevulinic acid an intermediate in porphyrin synthesis,[2] a fact confirmed by Dresel and Falk,[3] but also that the formation of protoporphyrin from δ-aminolevulinic acid occurs in a particle-free extract of duck erythrocytes.[4] The occurrence of the latter series of reactions in a soluble enzyme system[4,5] opened up the possibility of isolating the enzymes concerned with the individual steps and thus studying the reactions in greater detail. We have succeeded in isolating, from duck erythrocytes, a highly purified protein fraction containing only traces of hemoglobin which converts δ-aminolevulinic acid to the mono-pyrrole, porphobilinogen. The latter compound was isolated from the incubation mixture and characterized. While our work was in progress, Gibson, Neuberger and Scott[6] announced in a preliminary communication a similar preparation obtained from ox liver.

In a typical experiment, the protein fraction, obtained from about 350 ml. of washed red cells, containing approximately 15 mg. of protein-N, was dissolved in 20 ml. of 0.1 *M* phosphate buffer, *p*H 7.1, and incubated at 38° under nitrogen with 230 mg. of δ-aminolevulinic acid-5-C^{14} (0.018 mc./mM.) and 30 mg. of glutathione. The conversion of the substrate to porphobilinogen, during the incubation, was followed with Ehrlich reagent and after nine hours it appeared that about 110 mg. of porphobilinogen had been formed. Interference of glutathione with the Ehrlich reaction can be overcome by the addition of small amounts of cuprous oxide prior to the addition of the dimethylaminobenzaldehyde reagent. The incubation was terminated by the addition of trichloroacetic acid and from the supernatant fluid 58 mg. of crystalline porphobilinogen was isolated by a modification of the methods of Westall[7] and Cookson and Rimington.[8]

The porphobilinogen crystals were slightly pink in color and had the same form as those isolated from urine by Westall.[7] The material had a R_f of 0.51 on an ascending paper chromatogram in a butanol–acetic–water mixture, the same as that of a porphobilinogen sample[9] isolated from a pathological urine. The porphobilinogen was identified by spraying the paper with Ehrlich reagent or by its conversion to porphyrins on heating the paper in acetic acid vapor by the method of Westall. The latter conversion also supports the identity of the compound. Further evidence of the identity of the product which also supports the mechanism of its formation, was furnished by determining its radioactivity. Since the porphobilinogen is presumably synthesized from two moles of δ-aminolevulinic acid, its molar activity[10] should be twice that of the δ-aminolevulinic acid used as the substrate. The molar activities of the substrate, δ-aminolevulinic acid, and of the product, porphobilinogen, were found to be 242×10^3 c.p.m. and 487×10^3 c.p.m. respectively. This finding demonstrates experimentally the utilization of two moles of *d*-aminolevulinic acid for porphobilinogen formation. The product had the following elementary analysis: Calcd. for $C_{10}H_{14}O_4N_2 \cdot H_2O$: C, 49.2; H, 6.6; N, 11.5. Found: C, 49.3; H, 6.6; N, 11.4.

Further evidence that porphobilinogen is an intermediate in protoporphyrin synthesis was obtained by incubating equal volumes of a cell-free extract of duck erythrocytes[4] with equimolar amounts (0.01 mM.) of δ-aminolevulinic acid-5-C^{14} (0.018 mc./mM.) and with the above enzymatically synthesized radioactive porphobilinogen (0.036 mc./mM.) and subsequently isolating the hemin and determining its radioactivity. The radioactivities of the hemin samples synthesized from the δ-aminolevulinic acid and from the porphobilinogen were 92 c.p.m. and 85 c.p.m., respectively, after a two-hour incubation and 350 and 336 c.p.m. respectively after a fifteen-hour incubation period. This result is in agreement with the previous findings of Falk, *et al.*,[11] and with those of Bogorad and Granick.[5]

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(9) We wish to thank Dr. C. J. Waston for this sample.

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