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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(2) D. Shemin and C. S. Russell, THIS JOURNAL, **75**, 4873 (1953).

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(4) D. Shemin, T. Abramsky and C. S. Russell, THIS JOURNAL, **76**, 1204 (1954).

(5) L. Bogorad and S. Granick, *Proc. Nat. Acad. Sci.*, **39**, 1176 (1953).

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(8) G. H. Cookson and C. Rimington, *Biochem. J.*, **57**, 476 (1954).

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

(10) D. Shemin and S. Kumin, *J. Biol. Chem.*, **198**, 827 (1952).

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The enzyme fraction was prepared from washed duck erythrocytes by precipitation with ammonium sulfate. The protein fraction obtained by adjusting the ammonium sulfate concentration to 52% of saturation (*p*H 7.5) was purified by washing with half-saturated ammonium sulfate and by reprecipitations with ammonium sulfate at 50% saturation, *p*H 8. Finally the protein fraction insoluble in 35% saturated ammonium sulfate was discarded. Therefore the enzyme preparation represents the 35–50% ammonium sulfate fraction.

It is further of interest that whereas human red cells are incapable of forming heme *in vitro*, we have found that they contain the enzymes capable of converting δ-aminolevulinic acid to porphobilinogen.

DEPARTMENT OF BIOCHEMISTRY
COLLEGE OF PHYSICIANS AND SURGEONS — RUDI SCHMID[12]
COLUMBIA UNIVERSITY — DAVID SHEMIN
NEW YORK 32, N. Y.

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(12) Special Research Fellow, National Institutes of Health, U. S. Public Health Service.

RADIATION-CHEMICAL STUDIES WITH CYCLOTRON BEAMS[1]

Sir:

The use of cyclotron beams provides a powerful tool for studying the variation of radiation-chemical yields with ionization density—a datum of basic importance to the understanding of mechanisms of radiation effects, and to the interpretation of the effects of mixed radiations such as those present in nuclear reactors. The Brookhaven 60-inch cyclotron provides deuteron beams at 20 Mev. ($-dE/dX$ = 0.46 ev./Å. in H_2O) and alpha beams of 40 Mev. (1.88 ev./Å.). The energies may be reduced by absorbers so that rays having a great range of initial ionization densities can be introduced into solutions.

Figure 1 shows yields (G = molecules/100 ev.) of hydrogen gas from pure cyclohexane, and of ferric ion from 1 mM $FeSO_4$ solution in aerated aqueous 0.8 N H_2SO_4. The yields are plotted against the values of $-dX/dE$ (the reciprocal of the usual energy loss parameter) characterizing the particles as they enter the liquids. The dashed horizontal lines show the yields for the two reactions obtained with beams of 2 Mev. electrons from a Van de Graaff generator, for which $-dX/dE$ is very large; the yield curves should approach this value asymptotically. The ferrous sulfate curve includes yields reported for natural α-rays by McDonell and Hart,[2] and by Miller and Wilkinson.[3] Values near 6 are also reported by Lefort[4] and by Haissinsky and Anta.[5] The results show the expected continuous rise in G from the α-ray values to the electron yields. The α-ray and deuteron curves join together fairly well, although in theory a slight difference is expected in G for alphas and deuterons of the same initial energy loss parameter. By

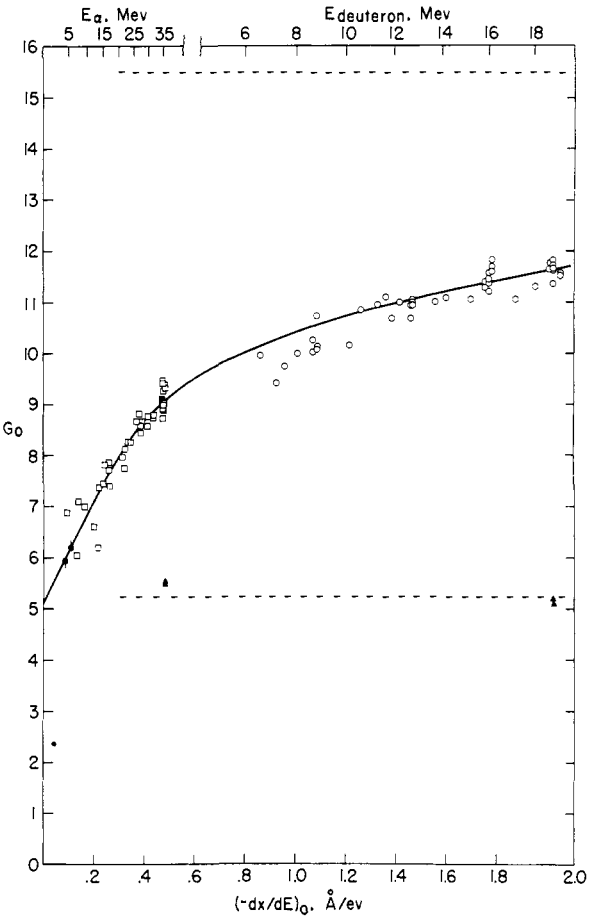

Fig. 1.—Radiation-chemical yields from deuterons and alpha particles: O, oxidation of $FeSO_4$ in air-saturated 0.8 N H_2SO_4 by deuterons; □, by cyclotron α's; ◐, by α's from dissolved Po (McDonell and Hart[2]); ●, by α's from an external Po source (Miller and Wilkinson[3]); ▲, hydrogen gas yields from pure cyclohexane by cyclotron alphas and deuterons. The dotted lines are the values of G found for $FeSO_4$ oxidation (15.5) and H_2 production in cyclohexane (5.25) by 2-Mev. electron beams from a Van de Graaff generator.

differentiation of the yields with respect to the initial beam energy, the instantaneous or "thin-target" value of G is found,[6] which at the highest deuteron energy used is only 13.3 and thus still lies well below the fast electron value of 15.5.

In contrast to aqueous solutions, the behavior of the hydrocarbon appears to be independent of the type of radiation. A great difference evidently exists in the mechanism of action of radiation on water and on hydrocarbons.

A detailed description of the above experiments and a discussion of the results are presently in preparation. Further work on the effects of ionization density on radiation-chemical reactions is in progress.

DEPARTMENT OF CHEMISTRY
BROOKHAVEN NATIONAL LABORATORY — ROBERT H. SCHULER
UPTON, LONG ISLAND, NEW YORK — AUGUSTINE O. ALLEN

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(1) Research carried out under the auspices of the U. S. Atomic Energy Commission.

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