135°, 171–171.5° or 197–199° (polymorphic forms), $[\alpha]_D$ + 164° (chl.), λ_{max}^{EtOH} 242 mμ (15,125). *Anal.* Calcd. for $C_{24}H_{34}O_6$: C, 68.87; H, 8.19. Found: C, 68.84; H, 8.15, and 11β,17α,20α,21-tetrahydroxy-2-methyl-4-pregnene-3-one 21-acetate (VII), m.p. 215–218.5°, $[\alpha]_D$ +67° (diox.). *Anal.* Calcd. for $C_{24}H_{36}O_6$: C, 68.54; H, 8.63. Found: C, 68.82; H, 8.60. The presence of the 17,20-glycol grouping in VII was shown by a negative Tollens test and periodic acid oxidation to 11β-hydroxy-2-methyl-4-androstene-3,17-dione, m.p. 206–208°, $[\alpha]_D$ +220° (chl.). *Anal.* Calcd. for $C_{20}H_{28}O_3$: C, 75.91; H, 8.92. Found: C, 75.98; H, 9.31. Hydrolysis of VI with potassium bicarbonate in methanol gave 11β,17α,21-trihydroxy-2-methyl-4-pregnene-3,20-dione (VIII), m.p. 237–238°, $[\alpha]_D$ +185° (95% EtOH), λ_{max}^{EtOH} 242 mμ (15,250). *Anal.* Calcd. for $C_{22}H_{32}O_5$: C, 70.18; H, 8.57. Found: C, 70.14; H, 8.61. N-Bromoacetamide in *t*-butyl alcohol–pyridine oxidized VI to 17α,21-dihydroxy-2-methyl-4-pregnene-3,11,20-trione 21-acetate (IX) in 72% yield, m.p. 205–209°, $[\alpha]_D$ +170° (acetone). *Anal.* Calcd. for $C_{24}H_{32}O_6$: C, 69.25; H, 7.75. Found: C, 68.94; H, 7.69.

Dehydration of the 11β-hydroxyl group of VI with thionyl chloride in pyridine afforded 17α,21-dihydroxy-2-methyl-4,9(11)-pregnadiene-3,20-dione 21-acetate (X), m.p. 220–223°, $[\alpha]_D$ +138° (chl.), λ_{max}^{EtOH} 240 mμ (16,750). *Anal.* Calcd. for $C_{24}H_{32}O_5$: C, 71.97; H, 8.05. Found: C, 72.05; H, 8.32. Practically quantitative conversion of X to 9α-bromo-11β,17α,21-trihydroxy-2-methyl-4-pregnene-3,20-dione 21-acetate (XI), [m.p. 125–130° dec., $[\alpha]_D$ +146° (chl.). *Anal.* Calcd. for $C_{24}H_{33}O_6Br$: Br, 16.07; Found: Br, 16.27, 16.06] was accomplished with N-bromoacetamide in *t*-butyl alcohol containing aqueous perchloric acid. XI with potassium acetate in acetone gave 9β,11β-epoxy-17α,21-dihydroxy-2-methyl-4-pregnene-3,20-dione 21-acetate (XII), 75% yield, m.p. 185–188°, $[\alpha]_D$ +49° (chl.). *Anal.* Calcd. for $C_{24}H_{32}O_6$: C, 69.20; H, 7.75. Found: C, 69.28; H, 7.90. Hydrofluoric acid converted XII to 9α-fluoro-11β,-17α,21-trihydroxy-2-methyl-4-pregnene-3,20-dione 21-acetate (XIII) in about 40% yield, m.p. 236–238°, $[\alpha]_D$ +167° (diox.), λ_{max}^{EtOH} 238.5 mμ (16,150). *Anal.* Calcd. for $C_{24}H_{33}O_6F$: C, 66.03; H, 7.62; F, 4.35. Found: C, 66.12; H, 7.31; F, 3.74. The corresponding 21-alcohol XIV, formed from XIII by potassium bicarbonate hydrolysis, melted at 250–253° dec., λ_{max}^{EtOH} 239 mμ (16,175). *Anal.* Calcd. for $C_{22}H_{31}O_5F$: C, 66.98; H, 7.92; F, 4.82. Found: C, 67.14; H, 7.97; F, 4.47. Oxidation of XIII with chromium trioxide in acetic acid produced 9α-fluoro-17α,21-dihydroxy-2-methyl-4-pregnene-3,11,20-trione 21-acetate (XV), m.p. 227–229°, λ_{max}^{EtOH} 235.5 mμ (15,500), $[\alpha]_D$ +167° (diox.). *Anal.* Calcd. for $C_{24}H_{31}O_6F$: C, 66.34; H, 7.19; F, 4.37. Found: C, 65.79; H, 7.23; F, 3.97.

Alkylation of 2-ethoxyoxalyl-11β,21-dihydroxy-4,17(20)-pregnadiene-3-one (III) with ethyl iodide, followed by removal of the ethoxyoxalyl grouping and acetylation gave 2-ethyl-11β,21-dihydroxy-4,17(20)-pregnadiene-3-one 21-acetate (XVI) in 9% yield, m.p. 149–151°, λ_{max}^{EtOH} 242 mμ (15,000). *Anal.* Calcd. for $C_{25}H_{36}O_4$: C, 74.96; H, 9.06. Found: C, 75.23; H, 9.17. Oxidation of XVI with hydrogen peroxide and osmium tetroxide produced 2-ethyl-11β,17α,21-trihydroxy-4-pregnene-3,-20-dione 21-acetate (XVII), m.p. 160–168°, isolated as a methanol solvate. *Anal.* Calcd. for $C_{25}H_{36}O_6 \cdot CH_3OH$: C, 67.21; H, 8.67. Found: C, 67.55; H, 8.97.

These compounds were tested in the Department of Endocrinology of the Upjohn Research Division. 2-Methylhydrocortisone acetate (VI) was found to be ten times as active as hydrocortisone in the glycogen deposition assay, while the corresponding 9α-fluoro derivative XIII was thirty-eight times as potent. In the salt retention assay VI and XIII were found to be more potent than DOCA by factors of two and six-tenths and ninety, respectively. More complete biological data will be published.[5]

The preparation of the 2-alkyl analogs of other steroid hormones will be reported at a later date.

The authors are indebted to J. L. Johnson, Mrs. G. S. Fonken and J. E. Stafford for infrared and ultraviolet absorption data, and to W. A. Struck and associates for microanalyses.

(5) W. W. Byrnes, L. E. Barnes, B. J. Bowman, W. E. Dulin, E. H. Morley and R. O. Stafford, *Proc. Soc. Exp. Med.*, in press.

RESEARCH LABORATORIES
THE UPJOHN COMPANY
KALAMAZOO, MICHIGAN

J. A. HOGG
F. H. LINCOLN
R. W. JACKSON
W. P. SCHNEIDER

RECEIVED NOVEMBER 15, 1955

AN EFFECT OF PYRIDOXAL-5-PHOSPHATE *IN VITRO* ON HEME SYNTHESIS AND CO_2 PRODUCTION FROM GLYCINE-2-C-14[1]

Sir:

Various species of vitamin B_6-deficient animals develop an anemia (dog,[2] pig,[3] rat,[4] duck[5]). The effect of pyridoxal-5-phosphate on heme synthesis was studied with duck blood since ducks have nucleated red cells which are able to synthesize labeled heme *in vitro* from glycine-2-C-14.[6]

Day-old Pekin ducklings were made vitamin B_6-deficient with a diet described by Hegsted and Rao.[5] After 8 days on the diet, the average weights of the control and deficient ducklings were 271 and 89 g., respectively. Two ml. samples of blood removed from the heart of each animal under ether anesthesia were incubated in Warburg vessels with glycine-2-C-14, in the presence and absence of pyridoxal-5-phosphate. The CO_2 released during the incubation was collected in 0.2 ml. of 10% ml. of 10% NaOH contained in the center well. After 2 hours the samples were chilled in ice and 3 ml. of rat blood was added to each vessel to increase the yield of heme. The cells were centrifuged and washed twice with 0.9% saline, and hemin was iso-

(1) This work was supported in part by a research grant (C-1852-(C 2)) from the National Institutes of Health, Public Health Service.

(2) P. J. Fouts, O. M. Helmer, S. Lepkovsky and T. H. Jukes, *J. Nutrition*, **16**, 197 (1938).

(3) H. Chick, T. F. Macrae, A. J. P. Martin and C. J. Martin, *Biochem. J.*, **32**, 2207 (1938).

(4) A. Kornberg, H. Tabor and W. H. Sebrell, *Am. J. Physiol.*, **143**, 434 (1945).

(5) D. M. Hegsted and M. N. Rao, *J. Nutrition*, **30**, 367 (1945).

(6) N. S. Radin, D. Rittenberg and D. Shemin, *J. Biol. Chem.*, **184**, 745 (1950).

lated by the procedure of Fischer.[7] The ratioactivity of each hemin sample was measured in duplicate. The radioactive CO_2 was precipitated and measured as $BaCO_3$ after the addition of a known amount of carrier sodium carbonate to each sample. All samples were counted in a windowless flow counter and the reported counts per minute were corrected to infinite thinness.

The results in Table I show that (1) heme is synthesized in the red cells of vitamin B_6-deficient ducklings from glycine-2-C-14 at a rate which is half or less than half of that found with control ducklings; (2) addition of pyridoxal-5-phosphate *in vitro* restores the ability of the deficient cells to synthesize heme at a normal rate; (3) there is no stimulatory effect of pyridoxal-5-phosphate on heme synthesis by normal duckling cells; and (4) the addition of pyridoxal-5-phosphate accelerates the conversion of glycine-2-C-14 to $C^{14}O_2$ in both normal and deficient red cells. Since hemolysates of duck cells also show a stimulation of pyridoxal-5-phosphate on heme synthesis, the observed effect is not dependent on the presence of intact cells.

TABLE I

HEME SYNTHESIS AND CO_2 PRODUCED BY THE INCUBATION OF GLYCINE-2-C-14 WITH DUCK BLOOD

Each vessel contained 2 ml. of heparinized blood from either vitamin B_6-deficient or control ducklings and 0.1 ml. of glycine-2-C-14 (23.5 μM.; 230,000 c.p.m./μM.). In addition, 1 mg. of crystalline pyridoxal-5-phosphate monohydrate in 0.1 ml. of saline was added to appropriate flasks and 0.1 ml. of saline was added to the others. Values given are the averages ± standard errors obtained from 4 deficient and 4 control ducklings. The p-values for 1, 2 and 3 are <0.01, >0.2 and <0.01, respectively, when calculated without regard to the paired nature of the data. When calculated by matched pair formula,[8] the p-values for the stimulatory effects of pyridoxal-5-phosphate are between 0.01 and 0.05 for 1 and 2 and less than 0.01 for 3.

	Hemin, c.p.m./mg.		C.p.m./total collected CO_2	
	No additions	Plus pyridoxal-5-phosphate	No additions	Plus pyridoxal-5-phosphate
Vitamin B_6-deficient	535 ± 83	1185[1] ± 147	2656 ± 616	3790[2] ± 520
Control	1309 ± 112	1256 ± 102	4370 ± 344	7478[2] ± 391

Results with succinate were similar to those found with glycine. The incorporation of sodium succinate-2,3-C-14 into heme was depressed in vitamin B_6-deficient duck blood whole cells and hemolysates, and stimulated by added pyridoxal-5-phosphate. However, δ-aminolevulinic acid-2,3-C-14 was incorporated equally well into the hemes of the B_6-deficient and control bloods and was not stimulated by added pyridoxal-5-phosphate. Since δ-aminolevulinic acid is a porphyrin precursor formed from glycine and succinate,[9,10] it appears that pyridoxal-5-phosphate acts specifically in the formation of δ-aminolevulinic acid from glycine and succinate.

(7) H. Fischer, *Org. Syntheses*, **21**, 53 (1941).

(8) A. L. Edwards, "Statistical Analysis," Rinehart and Co., Inc., New York, N. Y., 1946.

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

(10) A. Neuberger and J. J. Scott, *Nature*, **172**, 1093 (1953).

DEPARTMENT OF BIOCHEMISTRY MARTIN P. SCHULMAN
STATE UNIVERSITY OF N. Y. MEDICAL COLLEGE
SYRACUSE, NEW YORK DAN A. RICHERT

RECEIVED OCTOBER 28, 1955

DEGRADATION OF AMYLOPECTIN TO NIGEROSE

Sir:

Although the α-D (1→4) linkage is the principal glycosidic bond in amylopectin with branching occurring through α-D-glucopyranosidic (1→6) bonds, some linking at positions other than 4 and 6 is not excluded. Assuming complete reaction, the detection of a small amount of glucose by paper chromatography in the hydrolysate of periodate-oxidized (with subsequent reduction) amylopectin furnishes analytical evidence[1] for the presence of a small number of (1→3) or of both (1→2) and (1→4) linkages in amylopectin. We wish to present definitive evidence herein for the existence of an α-D-(1→3)-bond in amylopectin. This evidence consists of the isolation of nigerose (3-*O*-α-D-glucopyranosyl-D-glucose) as its crystalline β-D-octaacetate from an amylopectin hydrolysate produced under conditions which are known to minimize its formation by reversion to a negligible quantity.[2] A 0.4% solution of amylopectin (130 g., waxy maize starch) in 0.1 *N* hydrochloric acid, was hydrolyzed by heating at 97° to 67% completion. This hydrolysate, after removal of the acid by ion-exchange resin, was subjected to fractionation on a carbon (Nuchar C, unground) column by the general method of Whistler and Durso.[3] The fraction known to contain maltose and isomaltose was acetylated to give 40 g. of sirupy material from which most of the maltose was removed as β-maltose octaacetate by direct crystallization from ethanol, yield 18 g., m.p. 155–156°, $[\alpha]^{28}D$ +64° (*c* 4.5, chloroform). The material from the mother liquor was subjected to fractionation by silicate column extrusion chromatography and produced β-isomaltose octaacetate, 1.67 g., m.p. 144–146°, $[\alpha]^{30}D$ +98° (*c* 4.4, chloroform), and β-nigerose octaacetate, 350 mg., m.p. 140–145°, $[\alpha]^{25}D$ +80° (*c* 3.0, chloroform), X-ray powder diffraction pattern identical with that of known β-nigerose (sakébiose[4]) octaacetate[5] ("*y*-acetate"[6]). Upon further purification the melting point was 151–153°.

DEPARTMENT OF CHEMISTRY
THE OHIO STATE UNIVERSITY M. L. WOLFROM
COLUMBUS 10, OHIO A. THOMPSON[7]

RECEIVED OCTOBER 20, 1955

(1) M. Abdel-Akher, J. K. Hamilton, R. Montgomery and F. Smith, THIS JOURNAL, **74**, 4970 (1952).

(2) A. Thompson, M. L. Wolfrom and E. J. Quinn, *ibid.*, **75**, 3003 (1953).

(3) R. L. Whistler and D. F. Durso, *ibid.*, **72**, 677 (1950).

(4) K. Matsuda, G. Hiroshima, K. Shibasaki and K. Aso, *J. Fermentation Technol. (Japan)*, **32**, 498 (1954); *Tôhoku J. Agr. Research*, **5**, 239 (1954); *C. A.*, **49**, 8554 (1955).

(5) S. A. Barker, E. J. Bourne and M. Stacey, *J. Chem. Soc.*, 3084 (1953); S. Peat, W. J. Whelan and Kathleen A. Hinson, *Chemistry & Industry*, 385 (1955).

(6) A. Thompson, Kimiko Anno, M. L. Wolfrom and M. Inatome, THIS JOURNAL, **76**, 1309 (1954).

(7) Research Associate of the Corn Industries Research Foundation.

SYNTHESIS OF PHTHALIMIDINES FROM SCHIFF BASES AND CARBON MONOXIDE

Sir:

When a solution of 5 g. of benzaldehyde anil in 50 ml. of benzene was heated with 1 g. of dicobalt octacarbonyl catalyst[1] under 100–200 atmospheres pressure of carbon monoxide at 220–230° for 5–6 hours, 2-phenylphthalimidine (I), m.p. 263°, was obtained in 80% yield.

Anal. Calcd. for $C_{14}H_{11}ON$: C, 80.38; H,

(1) M. Orchin and W. C. Schroeder in P. H. Groggins, "Unit Processes in Organic Synthesis," McGraw-Hill Book Co., N. Y., 1952, Chapter 9.