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[3] ± 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.

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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°.

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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,

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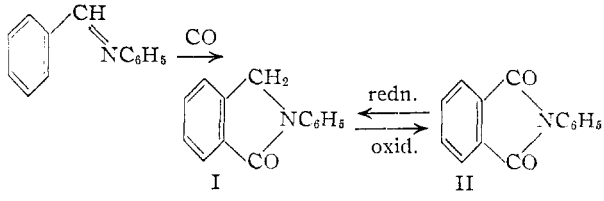
5.26; N, 6.70. Found: C, 80.26; H, 5.47; N, 6.92.

The structure was established by direct comparison with an authentic sample of 2-phenylphthalimidine (I) prepared by reduction of N-phenylphthalimide (II).[2] Compound I was also oxidized to II. Thus, a new synthesis of a phthalimidine has been demonstrated. This is the first example of a dicobalt octacarbonyl-catalyzed reaction in which carbon monoxide becomes attached to an aromatic nucleus.



I II

This reaction failed with nickel catalysts or when water or alcohol was present as the solvent.

In a similar way *p*-hydroxybenzaldehyde anil yielded 70% of 6-hydroxy-2-phenylphthalimidine, m.p. 215–216°.

Anal. Calcd. for $C_{14}H_{11}O_2N$: C, 74.67; H, 4.89; N, 6.22. Found: C, 74.88; H, 4.80; N, 6.42.

1-Naphthaldehyde anil afforded an analogous compound, $C_{18}H_{13}ON$, m.p. 177° in 96% yield. Two structures are possible for this compound as ring closure can occur at the 2- or 8-position. We believe the closure was at the 2-position to yield 2-phenylbenz[e]isoindolin-1-one (III) because the N-phenylnaphthalimide, m.p. 165°, formed on oxidation proved different from N-phenyl-1,8-naphthalimide, m.p. 202°, prepared from 1,8-naphthalenedicarboxylic acid.

Anal. Calcd. for $C_{18}H_{13}ON$: C, 83.38; H, 5.01; N, 5.40. Found (for III): C, 83.68; H, 4.98; N, 5.65; (for IV): C, 83.55; H, 5.05; N, 5.28.

2-Naphthaldehyde anil yielded 2-phenylbenz[f]-isoindolin-1-one (IV), m.p. 254°, in 80% yield. This structure was established by oxidation to N-phenyl-2,3-naphthalimide, m.p. 278°, identical with an authentic sample[3] prepared from 2,3-naphthalenedicarboxylic acid and aniline. The fact that ring closure occurred in the 3-position in this case is noteworthy since most ring closure reactions of 2-substituted naphthalene derivatives take place in the 1-position.

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(3) M. Freund and K. Fleischer, *ibid.*, **402**, 51 (1914).

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CATHOMYCIN. I. ISOLATION AND CHARACTERIZATION

Sir:

We have isolated in crystalline form a new antibiotic from broths of a new actinomycete. This antibiotic, designated cathomycin, shows clinical promise.[1]

The first papers on cathomycin from these laboratories were presented before the Annual Symposium on Antibiotics.[2] The production[3] of cathomycin by *Streptomyces spheroides*, the antimicrobial properties,[3–5] and the absorption and distribution in mice[6] have been reported. Cathomycin is highly effective for staphylococci resistant to other antibiotics.[3,4]

The isolation of the antibiotic from the broth was accomplished by the following steps. A crude residue from the filtered and evaporated broth was dissolved in water, and the solution was acidified to *ca.* *p*H 2. A precipitate formed which was separated and dried. The precipitate was triturated with acetone and the insoluble material was removed. The acetone solution was evaporated *in vacuo* and the residue was triturated with methanol. The insoluble material was removed, and the methanol filtrate was evaporated *in vacuo*. The methanol-soluble residue was triturated with petroleum ether which dissolved most of the dark-colored substances. The remaining residue was dissolved in dilute sodium hydroxide and then hydrochloric acid was added to cause precipitation. The dried precipitate was triturated repeatedly with ether, and the ether extract was evaporated. The amorphous residue crystallized from aqueous acetone or ethanol or mixtures of petroleum ether and acetone or ethanol.

Cathomycin is a pale yellow compound which has been obtained in two crystalline forms, one melting at 152–154° (most common), the other at 170–172°. It is optically active; $[\alpha]^{25}D$ −27° (*c* 1 in 1 *N* sodium hydroxide) and $[\alpha]^{25}D$ −44° (*C* 1 in pyridine).

Potentiometric titration in a mixture of water and acetone (3–4) showed two acidic functional groups, $pH_1\ ^1/_2$ *ca.* 4.7, equivalent weight 653, and $pH_2\ ^1/_2$ *ca.* 10, equivalent weight 660–680. Determination of acidic groups by the ultraviolet absorption method, gave values of $pH_1\ ^1/_2$, 3.8 and $pH_2\ ^1/_2$ 9.2.

The principal maxima in the ultraviolet absorption spectra of solutions are as follows: 307 mμ, $E^{1\%}_{1\ cm}$ 600 in 0.1 *N* sodium hydroxide; 324 mμ, $E^{1\%}_{1\ cm}$ 390 in 0.1 *N* hydrochloric acid–methanol; 304 mμ, $E^{1\%}_{1\ cm}$ 350 in *p*H 7 phosphate buffer.

The infrared spectra of the two crystalline forms are different. However, when the two forms are dissolved in acetone, followed by rapid precipitation with petroleum ether, the spectra of the precipitates are identical. The principal bands in the infrared spectra of the precipitates, examined as a Nujol mull, expressed in microns are: 5.8–6.0 (broad), 6.10, 6.21, 6.30, 6.49, 6.63, 7.4–7.6, (broad-shoulder), 7.78, 7.96, 8.27 (weak), 8.60 (shoulder), 8.7 (shoulder), 9.13, 9.40, 10.0–10.1 (broad), 10.28, 10.60 (broad), 12.0–12.30 (broad), 12.60–12.75 (broad), 13.07 and 13.39.

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