Biosynthesis of 5-Aminolevulinic Acid and Heme from 4,5-Dioxovalerate in the Rat

KATHRYN A. MORTON, JAMES P. KUSHNER, JAMES G. STRAKA, and BRUCE F. BURNHAM, *Department of Medicine, Division of Hematology-Oncology, University of Utah College of Medicine, Salt Lake City, Utah 84132*

ABSTRACT We previously demonstrated an alternate pathway for the biosynthesis of 5-aminolevulinic acid (ALA) in bovine liver mitochondria and of tetrapyrroles in suspensions of rat hepatocytes (1980. *J. Biol. Chem.* **255:** 3742; 1981. *Proc. Natl. Acad. Sci. USA.* **78:** 5335). This pathway involves a transamination reaction that incorporates the intact 5-carbon skeleton of 4,5-dioxovaleric acid (DOVA) into ALA.

We investigated this alternate pathway in vivo by the intraperitoneal injection of DOVA into rats. Incorporation of DOVA and [5-^{14}C]DOVA into urinary ALA and hepatic and erythroid heme was quantified and compared with the incorporation of [4-^{14}C]ALA and [2-^{14}C]glycine into heme.

Within 3 h of injection of 175 μmol of DOVA, urinary ALA excretion increased 2.4-fold over controls. After injection of [5-^{14}C]DOVA, 0.11% of the radioactivity was recovered as urinary ALA, which quantitatively accounted for the 2.4-fold increase in ALA excretion.

After the injection 175 μmol of [5-^{14}C]DOVA, 0.14% of the radioactivity was recovered after 3 h as hepatic heme. The injection of 1.75 mmol of [2-^{14}C]glycine or 175 μmol of [4-^{14}C]ALA resulted in recovery of 0.2 and 3.4%, respectively, of the radioactivity as hepatic heme after 3 h. These doses of radiolabeled DOVA, glycine, and ALA were injected into rats with phenylhydrazine-induced anemia. Recovery of radioactivity after 3 h as splenic (erythroid) heme was 0.35% for DOVA, 0.072% for glycine, and 0.25% for ALA.

These studies establish that the intact 5-carbon skeleton of DOVA can be incorporated into ALA and heme in vivo.

INTRODUCTION

5-Aminolevulinic acid (ALA)[1] is the first committed precursor in the biosynthesis of tetrapyrroles (1, 2). It has generally been accepted that the generation of ALA occurs exclusively by the condensation of succinyl coenzyme (Co)-A and glycine in a reaction catalyzed by the enzyme ALA synthase, EC 2.3.1.37 (ALA synthase) (1, 2) (Fig. 1A).

Recently several lines of evidence have suggested that in some biologic systems ALA may be synthesized by enzymic reactions that differ from the classical condensation reaction. For example, green plants require ALA for the biosynthesis of chlorophyll, but have no demonstrable ALA synthase activity (3, 4). They appear to synthesize ALA via a transamination reaction in which the intact 5-carbon skeleton of a precursor compound serves as the amino acceptor (5). Similar transamination reactions have also been demonstrated in algae and bacteria (6, 7). We recently reported the isolation and characterization of an enzyme from bovine liver mitochondria that synthesizes ALA by a transamination reaction (8, 9). This enzyme, L-alanine: 4,5-dioxovaleric acid aminotransferase, catalyzes a transamination reaction between L-alanine and 4,5-dioxovalerate (DOVA), yielding ALA and pyruvate (Fig. 1B). The capacity of the L-alanine: DOVA aminotransferase reaction to synthesize ALA appeared to exceed that of the ALA synthase reaction (8). Finally, we have presented in vitro evidence for the conversion of DOVA to porphyrins and heme in suspensions of intact, respiring rat hepatocytes (10).

In this report, we present evidence for the in vivo conversion of DOVA into ALA and heme in the rat.

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[1] *Abbreviations used in this paper:* ALA, 5-aminolevulinic acid; DOVA, 4,5-dioxovaleric acid.

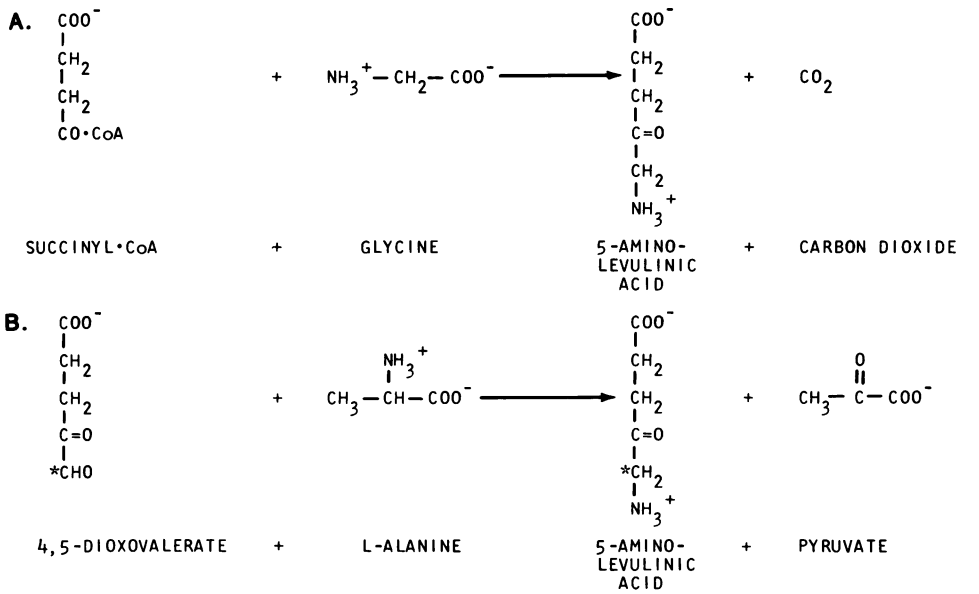

FIGURE 1 Formation of ALA. (A) The classical reaction catalyzed by the enzyme ALA synthase. In this reaction succinyl Co-A and glycine are condensed to form ALA and carbon dioxide. (B) The alternate reaction catalyzed by the enzyme L-alanine: DOVA aminotransferase. In this reaction the amino group of alanine is transferred to the intact 5-carbon skeleton of DOVA to yield ALA and pyruvate. The asterisk (*) identifies the position of the radiolabeled carbon in the [5-^{14}C]dioxovalerate used in this study. Generation of succinate by loss of this terminal carbon would result in loss of the radiolabel. Subsequent conversion of succinate into ALA by the classical condensation reaction would result in an unlabeled product.

METHODS

Materials. DOVA was prepared by the hydrolysis of dibromolevulinic acid (Porphyrin Products, Logan, UT) (8). A modification (8) of the method of Gnuchev et al. (11) was used to prepare [5-^{14}C]dibromolevulinic acid by generating diazo [^{14}C]methane from *N*-[*methyl*-^{14}C]methyl-*N*-nitroso-*p*-toluenesulfonamide (New England Nuclear, Boston, MA) (10). The specific activities of various preparations of [5-^{14}C]DOVA ranged from 5.7×10^3 to 1.25×10^5 dpm/μmol. New England Nuclear was the source of [4-^{14}C]ALA and [2-^{14}C]glycine. The ion-exchange resins AG 1-X8 and AG 50W-X4 were obtained in prepacked, stackable columns from Bio-Rad Laboratories, Richmond, CA.

Quantification of DOVA. Both radiolabeled and unlabeled DOVA were quantified by reaction with *o*-phenylenediamine as described by Jerzykowski et al. (12). DOVA excreted in urine was quantified as the benzoquinoxaline derivative by the method of Porra et al. (13).

Injection of rats. Male Sprague-Dawley rats weighing 280–300 g were obtained from the Simonson Laboratories, Gilroy, CA. The animals were placed in metabolic cages and allowed food and water ad lib. Rats were injected intraperitoneally with 5 ml of an azide-free balanced electrolyte solution, pH 6.8 (Isoton-II, Coulter Electronics Inc., Hialeah, FL), containing one of the following: 175 μmol of unlabeled or [5-^{14}C]DOVA, 1.75 mmol [2-^{14}C]glycine, 175 μmol [4-^{14}C]ALA, or no additives. Estimating total body water at 60% of body weight, these injections were expected to result in in vivo concentrations of 1 mM DOVA, 1 mM ALA, and 10 mM glycine. Urines were collected in 0.01 M HCl on ice.

Isolation and quantification of urinary ALA. Urine was adjusted to pH 7.0 with 3 N NaOH and then passed sequentially through prepacked stackable columns containing AG 1-X8 and AG 50W-X4. Porphobilinogen is adsorbed on AG 1-X8 and ALA is adsorbed on AG 50W-X4. The AG 50W-X4 was washed free of contaminating DOVA with 50 ml H_2O and the ALA was eluted by the method of Marver et al. (14). The eluted ALA was quantified spectrophotometrically after conversion to the 2-methyl-3-carbethoxy-4-(3-propionic acid) pyrrole by the method of Mauzerall and Granick (15). When radiolabeled ALA was employed, the specific activity of the isolated ALA was determined by liquid scintillation counting. Purity of the isolated radiolabeled ALA was verified by paper chromatography with two different solvent systems: phenol saturated with H_2O (R_f for ALA = 0.34) and *N*-butyl alcohol:glacial acetic acid:water (50:25:25, vol:vol:vol) (R_f for ALA = 0.45) (16).

Induction of hemolytic anemia. A 0.4% solution of filter sterilized phenylhydrazine was adjusted to pH 6.8 and intraperitoneal injections of 0.04 mg/g body wt were administered. Injections were repeated 24 and 32 h later; 144 h after the first injection, rats were used for experimentation. At this point reticulocyte counts averaged 30% and the spleen weights had increased fourfold. Histologic sections of the spleen revealed that erythroid precursors (normoblasts) were the predominant cells present.

Isolation and quantification of hepatic and erythroid heme. 3 h after intraperitoneal injections of either [5-^{14}C]DOVA, [2-^{14}C]glycine, or [4-^{14}C]ALA, rats were lightly anesthetized with ether. Livers were washed free of blood in situ after ligation of the inferior vena cava above the renal

vein and above the diaphragm. The liver was then perfused through the portal vein with 20 ml of cold Isoton II, carefully removed from the animal, washed again with cold Isoton II, minced, and homogenized. The spleens were removed from phenylhydrazine-treated animals, washed with cold Isoton II, minced, and homogenized.

Heme was extracted from the homogenates with 40 ml of ethyl acetate:glacial acetic acid (4:1, vol:vol). The ethyl acetate:glacial acetic acid was washed with 5-ml aliquots of cold H_2O and porphyrins were extracted into 1.5 N HCl. The ethyl acetate was taken to dryness and the heme redissolved in 20 ml of acetone:concentrated HCl (95:5, vol:vol). 2 g of talc were added, followed by the slow addition of 30 ml H_2O. This resulted in the adsorption of the heme onto the talc. The talc was then washed exhaustively with 0.01 M sodium acetate, pH 3.5, until no radiolabel could be detected in the washings. Heme was then eluted from the talc with acetone:concentrated HCl (95:5, vol:vol) and quantified as the pyridine hemochromogen (17). Heme radioactivity was determined by liquid scintillation counting. Quenching was avoided by decolorizing the heme solutions with H_2O_2 and waiting 24 h for the decay of chemiluminescence.

Purity of the isolated radiolabeled heme was verified by removal of the iron by the method of Grinstein (18). The protoporphyrin dimethyl ester was isolated by thin-layer chromatography on silica with a developing system of chloroform:petroleum ether (5:2, vol:vol) and eluted with dichloromethane:methanol (10:1, vol:vol). The protoporphyrin dimethyl ester was quantified spectrophotometrically and the radioactivity determined by liquid scintillation counting. The specific activity (disintegrations per minute per mole) of the protoporphyrin dimethyl ester was the same as that of the starting heme.

RESULTS

Verification of the chromatographic separation of ALA from DOVA. To verify our ability to separate ALA and DOVA in rat urine, 100 μmol of [5-^{14}C]DOVA (sp act 110 dpm/μmol) and 180 μmol of ALA were added to aliquots of acidified rat urine. The urine was neutralized and immediately passed through a prepacked column containing AG 50W-X4 (see Methods). The DOVA was not retained on the AG 50W-X4. The ALA was eluted from the AG 50W-X4 with 0.5 M sodium acetate and quantified as the Ehrlich chromophore. The recovery of ALA approximated 99% and there was no radioactivity in the eluant.

To verify that higher concentrations of DOVA were not retained on AG 50W-X4, [5-^{14}C]DOVA (sp act 7.5 × 10^5 dpm/μmol) was added to 4 ml of rat urine to give a final concentration of DOVA of 35 mM. This concentration was selected because of the observation that the intraperitoneal injection of 175 μmol of DOVA resulted in the rapid excretion of urine with a DOVA concentration of ~35 mM (see below). When urine containing 35 mM [5-^{14}C]DOVA was passed over the prepacked column containing AG 50W-X4, and the column washed once with 10 ml of H_2O, elution with 0.5 M sodium acetate recovered only 40 dpm above background levels (i.e., 0.006% of the counts applied). Counting of the resin itself showed no counts above background levels.

When rats were injected with [5-^{14}C]DOVA, radiolabeled compounds in addition to radiolabeled ALA and [5-^{14}C]DOVA appeared in the urine. These compounds, which were not identified, adhered weakly to AG 50W-X4 and were easily washed from the column with 50 ml H_2O. Radiolabeled ALA, which was eluted with 0.5 M sodium acetate, was verified by paper chromatography to be free of contamination by other radiolabeled compounds.

Urinary ALA excretion. Basal urinary ALA excretion, measured by pooling overnight urine collections from unperturbed (uninjected) rats, was 27 nmol ALA/3 h per rat (Table I).

After the injection of 5 ml of Isoton II, urinary ALA excretion increased to a mean value of 75 nmol ALA/rat in the first 3 h (Table I) and then rapidly returned to basal levels (mean = 28 nmol ALA/3h per rat; range 23.5–31.5; n = 24). Urinary ALA excretion was measured at 3–6-h intervals over the next 3 d. No evidence of a diurnal variation in urinary ALA excretion was observed. After the injection of 175 μmol of unlabeled DOVA in 5 ml of Isoton II, urinary ALA excretion increased to a mean value of 180 nmol/rat in the first 3 h (Table I) and then rapidly returned to basal levels. Most of the injected DOVA (75%) was excreted unmetabolized in the urine within 3 h (range 68–81%, n = 12).

After the injection of 175 μmol of [5-^{14}C]DOVA, 0.11% of the injected radioactivity was recovered as ALA (range 0.08–0.13%, n = 3). The specific activity of the recovered ALA was the same as that of the injected [5-^{14}C]DOVA.

Incorporation of [5-^{14}C]DOVA, [2-^{14}C]glycine, and [4-^{14}C]ALA into hepatic and erythroid heme. Radiolabeled compounds were injected intraperitoneally and 3 h later animals were killed and hepatic heme was extracted and quantified (see Methods). In rats

TABLE I
Urinary Excretion of ALA over a 3-H Period

Material injected	Number of rats studied	Urinary ALA	
		Mean value	Range
		nmol	
None	12	27	°
5 ml Isoton II	20	75‡	24–138
175 μmol DOVA	20	180‡	150–234

° Urines from the 12 control rats were pooled.
‡ Mean, 75; standard deviation, 8.98; variance, 0.11. Mean, 180; standard deviation, 13.3; variance, 0.07. 180 is greater than 75 with a P value < 0.001.

with phenylhydrazine-induced hemolytic anemia, erythroid heme was extracted from spleens and quantified (see Methods).

When 175 μmol of [5-^{14}C]DOVA were injected into normal rats 0.14% of the radioactivity was recovered as hepatic heme (Table II). This is equivalent to the incorporation of ~0.25 μmol of DOVA into hepatic heme. Since 8 μmol of DOVA are required (via conversion to ALA) for the synthesis of 1 μmol of heme, this is equivalent to the synthesis of 30.6 nmol of heme. When 1.75 mmol of [2-^{14}C]glycine was injected into unperturbed rats, 0.20% of the radioactivity was recovered as hepatic heme (Table II), equivalent to the incorporation of 3.5 μmol of glycine (or the synthesis of 437.5 nmol of heme). When 175 μmol of [4-^{14}C] ALA was injected into unpertubed rats, 3.4% of the radioactivity was recovered as hepatic heme (Table II), equivalent to the incorporation of 5.9 μmol of ALA (or the synthesis of 743 nmol of heme).

In rats with phenylhydrazine-induced hemolytic anemia, the injection of 175 μmol of [5-^{14}C]DOVA resulted in the recovery of 0.35% of the radioactivity in heme extracted from the erythropoietic spleens (Table II). This is equivalent to the incorporation of ~0.61 μmol of DOVA into erythroid heme (or the synthesis of 76.5 nmol of heme). When 1.75 mmol of [2-^{14}C]glycine was injected into phenylhydrazine-treated rats, 0.15% of the radioactivity was recovered as erythroid heme (Table II), equivalent to the incorporation of 0.26 μmol of ALA (or the synthesis of 32.8 nmol of heme).

DISCUSSION

These studies demonstrate that DOVA is incorporated into ALA and ultimately into hepatic and erythroid heme in vivo. This incorporation is presumably initiated by the mitochondrial enzyme L-alanine: DOVA aminotransferase (8).

Evidence that DOVA could be converted into ALA in vivo was first presented by Kissel and Heilmeyer (19). They injected unlabeled DOVA into rats and observed an increase in the excretion of ALA in the urine. Because unlabeled DOVA was used, it was not possible to conclude that the intact 5-carbon skeleton of DOVA had been incorporated into the ALA excreted in the urine. In our experiments [5-^{14}C]DOVA was used. The detection of the radiolabel in ALA (and heme) is strong evidence that the intact 5-carbon skeleton of DOVA has been incorporated into ALA via the transamination reaction we have previously described (Fig. 1B) (8, 9). Loss of the radiolabeled terminal aldehyde group of [5-^{14}C]DOVA would result in the generation of succinate. Were the succinate subsequently incorporated into ALA via the classical condensation, unlabeled ALA would be the product.

DOVA, with a carbonyl group three carbon atoms away from a carboxyl group, has the requisite properties expected for inhibitors of ALA dehydrase (20–22). Inhibition by DOVA of ALA dehydrase from *Clostridium tetanomorphum* has been demonstrated (23) and similar results have been obtained in our laboratory with ALA-dehydrase from bovine liver (unpub-

TABLE II

Incorporation of Radiolabeled Precursors into Hepatic and Erythroid Heme 3 H after Intraperitoneal Injection

Compound injected	Calculated concentration in total body water	% of injected radioactivity recovered as hepatic heme	% of injected radioactivity recovered as erythroid heme in rats treated with phenylhydrazine
	mM		
[5-^{14}C] DOVA 175 μmol Sp act 5.7 × 10^3 1.2 × 10^5 dpm/μmol	1	0.14 (range 0.11–0.16) *n* = 4	0.35 (range 0.26–0.43) *n* = 2
[2-^{14}C]glycine 1.75 mmol sp act 5.7 × 10^2 4.5 × 10^3 dpm/μmol	10	0.20 (range 0.25–0.24) *n* = 3	0.07 (range 0.068–0.075) *n* = 3
[4-^{14}C]ALA 175 μmol sp act 2.6 × 10^4 1.0 × 10^5 dpm/μmol	1	0.40 (range 2.0–5.1) *n* = 3	0.15 (range 0.13–0.17) *n* = 3

lished observation). In addition, studies using intact, respiring hepatocytes, erythroid cells, or *Rhodopseudomonas spheroides* have shown that porphyrin and heme synthesis is impaired by concentrations of exogenous DOVA as low as 0.1 mM. In the current whole-animal experiments, exogenous DOVA must traverse the cytosol, where ALA dehydrase is located, before entering the mitochondria. As a consequence, cytosolic DOVA concentration must be considerably higher than would be expected were DOVA synthesized in the mitochondria and directly converted to ALA.

Our finding that ALA excreted after the injection of [5-^{14}C]DOVA had the same specific activity as the injected DOVA is consistent with the direct conversion of DOVA to ALA. The excretion of ALA is consistent with the observation that DOVA is an inhibitor of ALA dehydrase. Under the conditions of our experiments, this inhibition must be incomplete as some radiolabeled heme was formed. Radiolabeled compounds that were not ALA, DOVA, or pyrroles were also detected in the urine after the injection of [5-^{14}C]DOVA. This finding is compatible with the observations of others that DOVA may have multiple metabolic fates (24–27).

Studies done in vitro with suspensions of rat hepatocytes demonstrated maximal incorporation of DOVA into tetrapyrroles when the concentration of DOVA in the medium was 0.5–1.0 mM (10). In an attempt to simulate these conditions in the whole animal, sufficient DOVA was injected to achieve a calculated concentration in total body water of 1 mM. The amount of radiolabeled ALA and glycine injected was calculated to approximate a concentration in cells in the range of the Michaelis constants of these substrates for ALA dehydrase and ALA synthase, respectively. These are rough approximations for several reasons. First, it is likely that the concentration of these compounds in portal blood (and probably in hepatocytes) is initially higher than the concentrations calculated for total body water. Second, these compounds are rapidly excreted in urine.

The quantity of the radiolabeled compounds injected in these experiments far exceeds the trace amounts used in past in vivo studies (28–32). This was done intentionally, so that isotope dilution by endogenous pools of glycine, ALA, and DOVA would be minimized. Thus, it is possible to compare the relative efficiency of conversion of these precursors into heme. In our studies the percentage of radiolabel recovered as hepatic heme after injection of [4-^{14}C]ALA was 17 times greater than that measured after the injection of [2-^{14}C]glycine (3.4% vs. 0.2%). After injection of [5-^{14}C]DOVA, 0.14% of the radiolabel was recovered as hepatic heme. These data indicate that under the conditions of these experiments, DOVA and glycine are converted to hepatic heme with similar efficiency. Previous in vitro studies suggested that the efficiency of conversion of DOVA to tetrapyrroles was greater than that of glycine (8). Failure to observe more efficient hepatic utilization of DOVA in the whole-animal experiments is unexplained but may reflect a combination of partial inhibition by DOVA of the cytosolic enzyme ALA dehydrase and differences in permeability between the cell and mitochondrial membranes.

In our studies of erythropoietic heme synthesis in the whole animal, DOVA appeared to be the most efficient substrate. Of the radiolabel injected as [5-^{14}C]DOVA, 0.35% was recovered as erythroid heme, compared with a recovery of 0.15% of the [4-^{14}C]ALA and 0.072% of the [2-^{14}C]glycine. The incorporation of labeled ALA into hepatic heme was greater than into erythroid heme (3.4% vs. 0.15%), a difference also observed by other investigators (33). It has been suggested that differences in membrane permeability to ALA between hepatic and erythroid cells is responsible for this observation (33).

These studies establish that the intact 5-carbon skeleton of DOVA can be incorporated into ALA and heme in vivo. To establish the physiologic significance of this alternate pathway in mammals will require proof that DOVA is present in mammalian mitochondria. This is an important point, as it has been suggested that L-alanine: DOVA aminotransferase activity is actually a secondary role of a mitochondrial enzyme whose normal function is to generate glycine from glyoxalate (34). Endogenous DOVA has been demonstrated in algae and bacteria (7, 35), and it has been suggested that in these organisms DOVA is derived from glutamate or 2-oxoglutarate. These putative precursors are naturally occurring compounds in mammalian mitochondria.

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REFERENCES

1. Burnham, B. F., and J. P. Kushner. 1981. Heme Biosynthetic Pathway. *In* Clinical Hematology. M. M. Wintrobe, G. R. Lee, D. R. Boggs, T. C. Bithel, J. Foerster, J. W. Athens, and J. N. Lukens, editors. Lea and Febiger, Philadelphia. 8th edition. 119–135.
2. Battersby, A. R., and E. McDonald. 1975. Biosynthesis of porphyrins, chlorins and corrins. *In* Porphyrins and Metalloporphyrins. K. M. Smith, editor. Elsevier, New York. 61–122.
3. Beale, S. I., S. P. Gough, and S. Granick. 1975. Biosynthesis of δ-aminolevulinic acid from the intact carbon skeleton of glutamic acid in greening barley. *Proc. Natl. Acad. Sci. USA.* **72:** 2719–2723.

4. Jurgenson, J. E., S. I. Beale, and R. F. Troxler. 1976. Biosynthesis of δ-aminolevulinic acid in the unicellular rhodophyte, *Cyanidium caldarium*. *Biochem. Biophys. Res. Commun.* **69:** 149–157.
5. Beale, S. I., and P. A. Castelfranco. 1973. ^{14}C Incorporation from exogenous compounds into δ-aminolevulinic acid by greening cucumber cotyledons. *Biochem. Biophys. Res. Commun.* **52:** 143–149.
6. Klein, O., D. Dornemann, and H. Senger. 1980. Two biosynthetic pathways to δ-aminolevulinic acid in algae. *Int. J. Biochem.* **12:** 725–728.
7. Bajkowski, A. S., and H. C. Friedmann. 1982. δ-Aminolevulinic acid formation: purification and properties of alanine: 4,5-dioxovalerate aminotransferase and isolation of 4,5-dioxovalerate from *Clostridium tetanomorphum*. *J. Biol. Chem.* **257:** 2207–2211.
8. Varticovski, L., J. P. Kushner, and B. F. Burnham. 1980. Biosynthesis of porphyrin precursors: purification and characterization of mammalian L-alanine: γ,δ-dioxovaleric acid aminotransferase. *J. Biol. Chem.* **255:** 3742–3747.
9. Varticovski, L., J. P. Kushner, and B. F. Burnham. 1980. Biosynthesis of porphyrin precursors: kinetic studies on mammalian L-alanine: γ,δ-dioxovaleric acid aminotransferase. *Int. J. Biochem.* **12:** 739–744.
10. Morton, K. A., J. P. Kushner, B. F. Burnham, and W. J. Horton. 1981. Biosynthesis of porphyrins and heme from γ,δ-dioxovalerate by intact hepatocytes. *Proc. Natl. Acad. Sci. USA.* **78:** 5335–6439.
11. Gnuchev, N. V., L. A. Neiman, and A. A. Poznanskaya. 1965. The synthesis of δ-aminolevulinic (5-^{14}C) acid and 4,5-dioxovaleric-(5-^{14}C) acid. *Biochemistry (Engl. Transl. Biokhimiya).* **30:** 138–140.
12. Jerzykowski, T., R. Winter, and W. Matuszewski. 1973. γ,δ-dioxovalerate as a substrate for the glyoxylase enzyme system. *Biochem. J.* **135:** 713–719.
13. Porra, R. J., O. Klein, D. Dornemann, and H. Senger. 1979. A simple method for the rapid determination of 4,5-dioxovaleric acid in the presence of 2-oxoglutarate and other 2-oxocarboxylic acids. *Hoppe-Seyler's Z. Physiol. Chem.* **361:** 187–190.
14. Marver, H. S., D. P. Tschudy, M. G. Perlroth, A. Collins, and G. Hunter, Jr. 1966. The determination of aminoketones in biological fluids. *Anal. Biochem.* **14:** 53–60.
15. Mauzerall, D., and S. Granick. 1956. The occurence and determination of δ-aminolevulinic acid and porphobilinogen in urine. *J. Biol. Chem.* **219:** 435–446.
16. Fink, K., R. E. Cline, and R. M. Fink. 1963. Paper chromatography of several classes of compounds: correlated R_f values in a variety of solvent systems. *Anal. Chem.* **35:** 389–398.
17. Fuhrhop, J.-H., and K. M. Smith. 1975. Laboratory methods. *In* Porphyrins and Metalloporphyrins. K. M. Smith, editor. Elsevier/North Holland, New York. 757–869.
18. Grinstein, M., 1947. Studies of protoporphyrin. XIII. A simple and improved method for the preparation of pure protoporphyrin from hemoglobin. *J. Biol. Chem.* **167:** 515–519.
19. Kissel, H. J., and L. Heilmeyer, Jr. 1969. Detection and quantitative determination of γ,δ-dioxovaleric acid and δ-aminolevulinic acid in rats. *Biochim. Biophys. Acta.* **177:** 78–87.
20. Nandi, D. L., and D. Shemin. 1968. δ-Aminolevulinic acid dehydratase of *Rhodopseudomonas spheroides*. III. Mechanisms of porphobilinogen synthesis. *J. Biol. Chem.* **243:** 1236–1242.
21. Nandi, D. L., K. F. Baker-Cohen, and D. Shemin. 1968. δ-Aminolevulinic acid dehydratase of *Rhodopseudomonas spheroides*. I. Isolation and properties. *J. Biol. Chem.* **243:** 1224–1230.
22. Tschudy, D. P., R. A. Hess, and B. C. Frykholm. 1981. Inhibition of δ-aminolevulinic acid dehydrase by 4,6-dioxoheptanoic acid. *J. Biol. Chem.* **256:** 9915–9928.
23. Brumm, P. J., G. A. Thomas, and H. C. Friedmann. 1982. The role of 4,5-dioxovaleric acid in porphyrin and vitamin B-12 formation by Clostridia. *Biochem. Biophys. Res. Commun.* **104:** 814–822.
24. Nemeth, A. M., C. S. Russell, and D. Shemin. 1957. The succinate-glycine cycle. II. Metabolism of δ-aminolevulinic acid. *J. Biol. Chem.* **229:** 415–422.
25. Wang, F. K., J. Koch, and E. L. R. Stockstad. 1970. Metabolism of 5-hydroxy-4-keto-valeric acid in the rat. *Biochem. Biophys. Res. Commun.* **40:** 576–582.
26. Braunstein, A. E., A. A. Poznanskaya, R. A. Spryshkova, and N. V. Gnuchev. 1964. Incorporation of the C-5 carbon atom of δ-aminolevulinic acid into inosinic acid and hypoxanthine in pigeon liver. *Dokl. Akad. Nauk S.S.S.R.* **157:** 982–984.
27. Shigesada, K. 1972. Possible occurrence of a succinate-glycine cycle in *Rhodospirillum rubrum*. *J. Biochem.* **71:** 961–972.
28. Berlin, J. I., A. Neuberger, and J. J. Scott. 1956. The metabolism of δ-aminolevulinic acid. I. Normal pathways, studied with the aid of ^{15}N. *Biochemistry.* **64:** 80–90.
29. Dowdle, E., P. Mustard, N. Spong, and L. Eales. 1968. The metabolism of [5-^{14}C] δ-aminolaevulinic acid in normal and porphyric human subjects. *Clin. Sci. (Oxf.).* **34:** 233–251.
30. Robinson, S. H., M. Tsong, B. W. Brown, and R. Schmid. 1966. Sources of bile pigment in rat: studies of "early-labeled" fraction. *J. Clin. Invest.* **45:** 1569–1584.
31. Robinson, S. H., R. Lester, J. F. Crigler, Jr., and M. Tsong. 1967. Early-labeled peak of bile pigment in man: studies with glycine-^{14}C and delta-aminolevulinic acid-^{3}H. *N. Engl. J. Med.* **277:** 1323–1329.
32. Levitt, M., B. A. Schacter, A. Zipursky, and L. G. Israels. 1968. The non-erythropoietic component of early bilirubin. *J. Clin. Invest.* **47:** 1281–1294.
33. Robinson, S. H. 1972. Formation of bilirubin from erythroid and nonerythroid sources. *Semin. Hematol.* **9:** 43–53.
34. Noguchi, T., and R. Mori. 1981. Biosynthesis of porphyrin precursors in mammals. Identity of alanine: γ,δ-dioxovalerate aminotransferase with alanine: glyoxylate aminotransferase. *J. Biol. Chem.* **256:** 10335–10339.
35. Porra, R. J., O. Klein, D. Dornemann, and H. Senger. 1980. A fast and convenient assay for 4,5-dioxovaleric acid in biological systems. *Int. J. Biochem.* **12:** 735–737.