166° (*Anal.* Found: C, 77.69; H, 11.31). The di-3,5-dinitrobenzoate of I melts at 192–194°, $[\alpha]^{20}D$ +29° (*Anal.* Found: C, 60.80; H, 6.00; N, 6.73), and of II melts at 176–178°, $[\alpha]^{20}D$ +19° (*Anal.* Found: C, 61.17; H, 6.42; N, 6.97). We prepared the diacetate of III, m.p. 68–69°, $[\alpha]^{20}D$ −3° (*Anal.* Found: C, 74.02; H, 9.86), and of IV, m.p. 91–93°, $[\alpha]^{20}D$ −1° (*Anal.* Found: C, 73.90; H, 10.05). The dibenzoate of III melts at 96–98°, $[\alpha]^{20}D$ +3° (*Anal.* Found: C, 78.37; H, 8.85), and that of IV at 138–140°, $[\alpha]^{20}D$ + 2° (*Anal.* Found: C, 78.33; H, 8.58). Moreover, the acid-catalyzed rearrangement of pseudosarsasapogenin and of pseudosmilagenin led quantitatively to sarsasapogenin and to smilagenin, respectively.

We were able to isolate from the mixture resulting from the oxidation of pseudosarsasapogenin the dextrorotatory α-methylglutaric acid,[6] $[\alpha]^{20}D$ +18° (EtOH), m.p. 78.5–81° (*Anal.* Found: C, 49.40; H, 6.76). From pseudosmilagenin we obtained the enantiomorphic levorotatory α-methylglutaric acid, $[\alpha]^{20}D$ −20° (EtOH), m.p. 78.5–81° (*Anal.* Found: C, 49.79; H, 7.03). A mixture of these isomers melted at 66–68°. Comparison of the infrared absorption spectra of these compounds with an authentic sample of α-methylglutaric acid showed all three to be identical.

Finally, the selective tosylation of the primary hydroxyl group at C-26 and subsequent reduction with lithium aluminum hydride converted dihydrosarsasapogenin and dihydrosmilagenin to the identical 16,22-epoxycoprostan-3β-ol, $[\alpha]^{20}D$ −4°, m.p. 137–139°; *Anal.* Found: C, 80.39; H, 11.73. The benzoate melts at 138–140°, $[\alpha]^{20}D$ +1°; *Anal.* Found: C, 80.57; H, 10.27. Thus it appears established that the isomerism of sarsasapogenin and smilagenin rests in the asymmetry at C-25.

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## ON THE MECHANISM OF THE ENZYMATIC SYNTHESIS OF GLUTATHIONE

*Sir:*

The synthesis of GSH[1] from L-γ-glutamyl-L-cysteine, glycine and ATP by a pigeon liver enzyme has been shown to involve the liberation of an equivalent amount of $P_i$.[2] Recently the enzyme which catalyzes the same reaction has been isolated from brewer's yeast autolysate and purified 1,500-fold.[3] Using the purified yeast enzyme, the quantitative changes in nucleotides as well as the $P_i$ liberated during GSH synthesis have now been determined. As is shown in Table I, GSH synthesis is accompanied by the liberation from ATP of an equivalent amount of both $P_i$ and ADP. The over-all reaction may hence be formulated as

L-γ-glutamyl-L-cysteine + glycine + ATP ⟶ GSH + ADP + $P_i$ (1)

In the course of attempts to elucidate the detailed mechanism of GSH synthesis, the exchange of phosphate residues between ADP and ATP was tested in the same system. $P^{32}$-ATP was isolated from a rabbit which had been injected with $P^{32}$-orthophosphate. Incubation of the radioactive ATP with AMP and muscle myokinase yielded $P^{32}$-ADP which was purified on a Dowex-1 column,[4] and isolated as the barium salt. As shown in Table II, low concentration of the yeast enzyme effects a transfer of the phosphate from ATP to ADP.

TABLE I

BALANCE STUDY OF GSH SYNTHESIS

The reaction mixture, 20.0 ml., contained 0.01 *M* tris buffer, 0.005 *M* KCN, 0.004 *M* $MgSO_4$, 0.005 *M* $C^{14}$-glycine, 0.002 *M* glutamylcysteine, 0.015 mg. of yeast enzyme per ml. and 0.4% bovine serum albumin. Incubation was 60 min. at 37°, *p*H 8.5. GSH was determined by isotopic assay,[5] $P_i$ by the method of Gomori,[6] and the nucleotides by Siekevitz and Potter's modification,[7] of the Dowex-1 technique of Cohn and Carter.[4] The results are expressed in μM per ml. of reaction mixture.

| | GSH Synthesized | $P_i$ | AMP | ADP | ATP |
|---|---|---|---|---|---|
| Initial | 0 | 0 | 0.04 | 0.45 | 1.18 |
| Final | 0.48 | 0.49 | .04 | .89 | 0.71 |
| Δ | + .48 | + .49 | 0 | + .44 | − .47 |

TABLE II

PHOSPHATE EXCHANGE BETWEEN RADIOACTIVE ADP AND ATP

The reaction mixture, 3.0 ml., contained 0.01 *M* tris buffer, 0.005 *M* KCN, 0.004 $MgSO_4$, $3.12 \times 10^{-3}$ *M* $P^{32}$-ADP, $2.53 \times 10^{-3}$ *M* ATP, and 0.1% bovine serum albumin. Incubation was 60 min. at 37°, *p*H 8.5. Nucleotides were separated and assayed by the Dowex-1 technique.[4]

| Enzyme, mg./ml. | ADP, c.p.m./μM. | ATP, c.p.m./μM. | Exchange, % |
|---|---|---|---|
| ... | 2370 | 39 | .. |
| 0.001 | 2280 | 400 | 29 |
| .003 | 1530 | 1280 | 92 |
| .030 | 1440 | 1380 | 99 |

Since AMP was not formed and since concentrations of ATP and ADP remained unchanged under these conditions it may be concluded that the observed exchange is not due to myokinase action. The exchange data may be interpreted as evidence for the reversible phosphorylation of the enzyme.

Enzyme + ATP ⟶ Enzyme-P + ADP (2)

Enzyme-P + $P^{32}$-ADP ⟶ Enzyme + $P^{32}$-ATP (3)

In view of the highly purified state of the enzyme, it appears highly probable that the exchange of phosphate between ATP and ADP is effected by the same enzyme which is responsible for GSH synthesis. Furthermore reactions 2 and 3 are consistent with the known powerful inhibition of GSH

(1) The following abbreviations have been used: GSH, glutathione; ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; Tris, tris-(hydroxymethyl) aminomethane.

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synthesis by ADP.[8] It may therefore be proposed that in the synthesis of GSH from glutamylcysteine and glycine, phosphorylation of the enzyme by ATP is the initial step.

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## δ-AMINOLEVULINIC ACID, ITS ROLE IN THE BIOSYNTHESIS OF PORPHYRINS AND PURINES[1]

*Sirs:*

We wish to report our finding that δ-aminolevulinic acid (II) can replace the two substrates, "active" succinate[2,3,4] and glycine[5,6] for porphyrin synthesis. It would appear, therefore, that II is the source of all the atoms of protoporphyrin as outlined in Fig. 1.

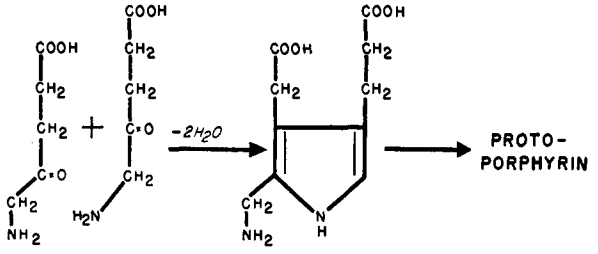


Fig. 1.—The formation of the precursor pyrrole for porphyrins from two moles of α-aminolevulinic acid.

The radioactivity of hemin obtained from either $C^{14}$ glycine plus unlabeled succinate or $C^{14}$ succinate plus unlabeled glycine, in duck blood, is reduced by 80 to 90% by the addition of an equimolar amount of unlabeled II. Also, II labeled with $N^{15}$ or with $C^{14}$ in the δ-carbon atom formed heme containing the isotope in many times the concentration of that formed from labeled glycine; hemin, synthesized from 0.05 mM. of $C^{14}$-labeled II and from 0.05 mM. of $C^{14}$-labeled glycine of equal activity had an activity of 22,000 c.p.m. and 500 c.p.m., respectively. II is the decarboxylated product of α-amino-β-ketoadipic acid (I), which can be formed from a condensation of "active" succinate and glycine. The above finding is evidence that I is an obligatory intermediate in the process (Figs. 1 and 2). The formula of the precursor pyrrole (Fig. 1), suggested on theoretical grounds, is the same as that proposed[7,8,9] for porphobilinogen.

II, which has not been previously described, was synthesized by three different procedures: (1) by exhaustive benzoylation of imidazole propionic acid[10] followed by hydrolysis, (2) by nitrosation of β-ketoadipic acid followed by reduction and (3) by a phthalimide synthesis on δ-chlorolevulinic acid.

Other metabolic pathways for II seem probable: for instance, a route by which the α-carbon atom of glycine may be utilized for the ureido carbon atoms of the purines,[11] for the β-carbon atom of serine,[12,13] etc., since II may be considered to be a derivative of the α-carbon atom of glycine. Testing the implications of Fig. 2 we have found that the δ-carbon atom of II is incorporated into the ureido groups of purines in much higher concentration than is the α-carbon atom of glycine in comparable experiments. This accords with the key steps in the postulated cycle (Fig. 2).

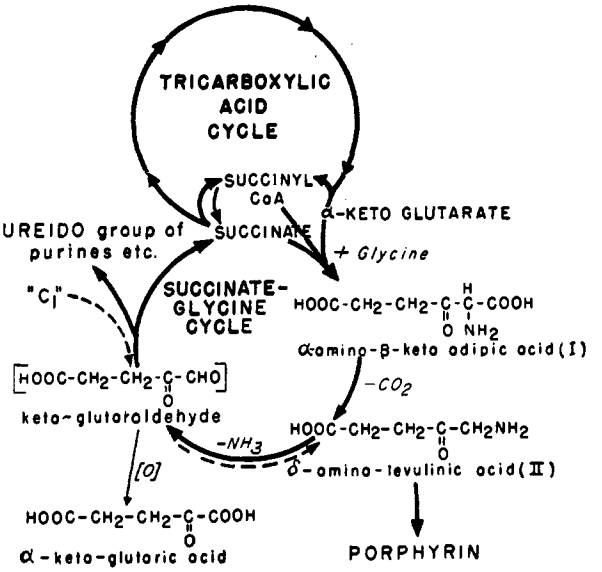


Fig. 2.—Succinate–glycine cycle: a metabolic pathway for the oxidation of glycine.

Further, this succinate–glycine cycle geared with the Kreb's citric acid cycle may account for the serine–glycine reaction,[12,13,14] may explain the metabolic reactions of the "one-carbon atom" compounds and provide via the oxidation of the proposed ketoglutaraldehyde to ketoglutaric acid, a pathway by which succinate can be converted to ketoglutarate. Consistent with the possible reversibility of these reactions outlined in Fig. 2 is our earlier finding that isotopic formate is utilized,

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