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*PATHWAY OF SERINE FORMATION FROM CARBOHYDRATE IN RAT LIVER**

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There are a number of reports on the formation of serine from glucose in animal tissues,[1-3] but experimental evidence for the steps in the main pathway of serine formation has not been available up to now. Sallach[4] has recently reported the presence of a transaminase between 3-hydroxypyruvic acid and alanine and has presented a possible pathway of serine formation from D-glyceric acid through 3-hydroxypyruvic acid. This paper reports the formation of serine from glucose, D-glyceric acid, 3-phosphoglyceric acid, and 3-phosphohydroxypyruvic acid by a rat-liver enzyme system and presents an alternative pathway of serine formation.

EXPERIMENTAL

Preparation of Enzyme System.—The livers from male rats, weighing 250–300 gm., were homogenized with 2 volumes of 0.1 *M* phosphate buffer (pH 7.4) in a Waring Blendor for 40 seconds. The homogenate was then centrifuged at 600 × g for 10 minutes and the supernatant fluid recentrifuged at 105,400 × g for 30 minutes in the Spinco preparative ultracentrifuge. The supernatant fluid from this was fractionated with saturated $(NH_4)_2SO_4$, and the enzyme activity was found in the fraction precipitating between 33–66 per cent saturation. This was dissolved in 0.1 *M* phosphate buffer and dialyzed against 0.03 *M* phosphate buffer, both at pH 7.4, for 6 hours. This served as the enzyme system. When inorganic phosphate determination was required, 0.2 *M* Tris buffer (pH 7.4) was substituted. All isolation procedures were carried out in the cold (<3° C.).

Radioactive DL-glyceric acid was purchased from Isotopes Specialties Co., Glendale, California, and pyruvamide and zinc lactate from Tracerlab, Inc. Radioactive glucose was generously furnished by Dr. W. Z. Hassid, radioactive 3-phosphoglyceric acid by Dr. J. A. Basham, and unlabeled 3-phosphohydroxypyruvic acid by Dr. C. E. Ballou.[5]

Assay Methods.—One milliliter of the enzyme preparation, the indicated substrates and cofactors, and 2 ml. of buffer solution in a total volume of 5 ml. were incubated for 2 hours at 37° C. in Warburg vessels. The reaction was stopped by the addition of trichloroacetic acid to give a final concentration of 10 per cent.

When radioactive substrates were employed, the serine formed was determined by adding carrier serine and recrystallizing to constant specific activity according

to the method of Siekevitz and Greenberg.[6] The serine formed from nonradioactive substrates was determined by periodate oxidation and measurement of the formaldehyde, according to the method of Frisell *et al.*[7] Inorganic phosphate was measured by the method of Lowry and Lopez.[8]

RESULTS

Serine Formation from Uniformly Labeled Glucose.—Table 1 shows the formation of serine from uniformly labeled glucose and the considerable dilution of the radioactivity in the serine by the addition of nonradioactive 3-phosphoglyceric acid. The highest results were obtained anaerobically and in the presence of NaF, which would preclude the reaction proceeding to pyruvic acid. The tissue with the highest activity of serine formation was liver (Table 2).

TABLE 1

SERINE FORMATION FROM UNIFORMLY LABELED GLUCOSE INCUBATIONS WITH 66.8×10^4 C.P.M. OF RADIOGLUCOSE

Atmosphere	Addition	Serine (c.p.m.)	Respiratory CO_2 (c.p.m.)
O_2	None	360	255
N_2	None	560	75
O_2	5×10^{-3} *M* NaF	160	138
N_2	5×10^{-3} *M* NaF	960	68
N_2	NaF, 200 μM PGA*	313	...

* PGA = Nonradioactive 3-phosphoglyceric acid.

TABLE 2

ACTIVITY OF SERINE FORMATION OF VARIOUS TISSUES*

Tissue	Serine (c.p.m.)
Liver	1,740
Kidney	460
Spleen	0

* Each flask contained 66.8×10^4 c.p.m. of radioglucose, 400 mg. of tissue homogenate, 5×10^{-3} *M* NaF, and 2 ml. of 0.1 *M* phosphate buffer (pH 7.4) in 5 ml. anaerobic incubation.

Serine Formation from Pyruvic Acid-2-C^{14} and Lactic Acid-2-C^{14}.—Although it has been reported that serine was synthesized from pyruvic acid,[9] neither pyruvamide-2-C^{14} (16×10^5 c.p.m.) nor lactic acid-2-C^{14} (46×10^5 c.p.m.) yielded any appreciable serine with our enzyme, and this negative result was not altered by addition of either ATP, KCl, or DPN.

Serine Formation from Uniformly Labeled and Nonradioactive 3-Phosphoglyceric Acid.—The dilution of the radioactive serine by nonradioactive 3-phosphoglyceric acid in the presence of NaF (Table 1) suggested that serine might be formed from 3-phosphoglyceric acid without passing through pyruvic acid. This possibility was tested with both uniformly labeled and nonradioactive 3-phosphoglyceric acid. It was found that 3-phosphoglyceric acid was converted to serine and, moreover, that the reaction was accelerated by DPN and alanine or glutamic acid (Tables 3 and 4). No difference was found in the stimulation of serine formation by these two amino acids in these crude enzyme preparations.

Serine Formation from D-*Glyceric Acid-3-C^{14}.*—Although it was reported that D-glyceric-1-C^{14} acid gives considerable amounts of serine,[4] it was found that the

conversion of D-glyceric acid to serine was only slight without ATP but was significant in the presence of ATP, DPN, and alanine (Table 5).

TABLE 3

SERINE FORMATION FROM UNIFORMLY LABELED 3-PHOSPHOGLYCERIC ACID*

Additions	Serine (c.p.m.)	Recovery (Per Cent)
None	10,253	2.0
Glutamate (20 μM)	25,628	5.2
DPN (400 μgm.)	19,254	3.8
ATP (20 μM)	18,434	3.6
Glutamate, DPN	33,238	6.6
Glutamate, DPN, ATP	33,596	6.7

* Anaerobic incubations with 5×10^5 c.p.m. of uniformly labeled 3-phosphoglyceric acid and 5×10^{-3} *M* NaF.

TABLE 4

SERINE FORMATION FROM NONRADIOACTIVE 3-PHOSPHOGLYCERIC AND 3-PHOSPHOHYDROXY PYRUVIC ACID

Additions	Serine (μM)	Inorganic P (μM)	Recovery (Per Cent)
PGA (5 μM)*, †	0	0.5	0
PGA, DPN (400 μgm.)	0.52	...	10.4
PGA, DPN, alanine (7.5 μM)†	0.66	1.37	13.2
PGA, DPN, alanine, NaF (5×10^{-3} *M*)	0.43	...	8.0
PGA, alanine	0.10	...	2.0
PGA, DPN, alanine, ATP (20 μM)	0.67	...	13.2
3-P-Pyr (3 μM)‡	1.08	...	36.0
3-P-Pyr, alanine (7.5 μM)	1.23	...	41.0

* PGA = 3-phosphoglyceric acid.
† 0.2 *M* Tris buffer (pH 7.4).
‡ 3-P-Pyr = 3-phosphohydroxypyruvic acid.

TABLE 5

SERINE FORMATION FROM DL-GLYCERIC ACID-3-C^{14}*

Additions	Serine (c.p.m.)	Recovery (Per Cent)
None	2,865	0.5
ATP (20 μM)	21,642	4.0
DPN (400 μgm.)	4,858	0.8
Alanine (20 μM)	5,865	1.1
Alanine, DPN	8,129	1.6
Alanine, DPN, ATP	60,750	12.1

* Incubations with 5×10^5 c.p.m. of DL-glyceric acid-3-C^{14}.

Serine Formation from 3-Phosphohydroxypyruvic Acid.—When 3-phosphohydroxypyruvic acid was incubated with the enzyme, serine synthesis was observed, and this was accelerated by the addition of alanine (Table 4).

DISCUSSION

It is a well-known fact that serine and glycine are readily interconvertible,[10-12] but, from the viewpoint of actual serine synthesis in animal tissues, it appears that certain three-carbon compounds, derived from glycolysis, are more likely to be major precursors of serine.[13] Anker suggested the intermediary formation of serine from pyruvate on the pathway to glycine,[9] but our results do not support this suggestion. Sallach presented a possible scheme which shows serine formation from D-glyceric acid through 3-hydroxypyruvic acid.[4] As mentioned above, D-glyceric acid itself was a poor source and required DPN, alanine, and especially ATP to produce good results. These findings suggest that D-glyceric acid may be

converted to serine through 3-phosphoglyceric acid. As shown in Tables 3 and 4, 3-phosphoglyceric and 3-phosphohydroxypyruvic acid are converted to serine by the additions of DPN and alanine, and alanine, respectively. Although ATP showed a slight activation on the formation of serine from 3-phosphoglyceric acid, it is likely that this is due to the protection of 3-phosphoglyceric acid from dephosphorylation.

On the basis of the above-cited results we present the following alternative pathway of serine biosynthesis (Fig. 1). This pathway might involve the following

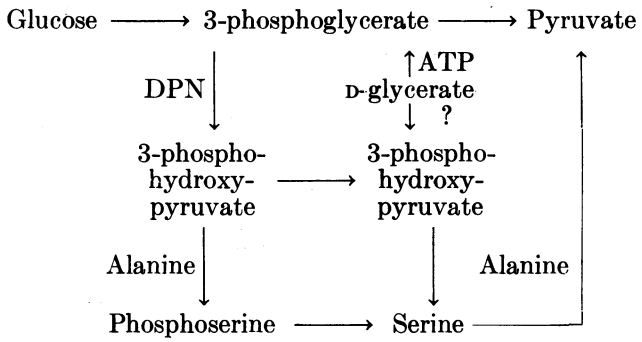

FIG. 1.—Possible pathway of serine formation.

enzymes: a glyceric acid kinase, a 3-phosphoglyceric acid dehydrogenase, a 3-phospho- or hydroxypyruvic acid transaminase, and a phosphatase. From Figure 1 it is seen that one point which still remains unsettled is the identity of the precursor of serine, 3-hydroxypyruvic acid, or phosphoserine. We hope to determine this in the near future.

SUMMARY

1. It was found that a crude enzyme system of rat liver can catalyze serine formation from glucose, D-glyceric, 3-phosphoglyceric, and 3-phosphohydroxypyruvic acids.

2. It was necessary to add ATP, DPN, and alanine to secure synthesis from D-glyceric acid; DPN and glutamate or alanine from 3-phosphoglyceric acid; and alanine from 3-phosphohydroxypyruvic acid.

3. A possible pathway of serine biosynthesis is presented and discussed.

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[5] The synthesis of this compound by Dr. C. E. Ballou, of the Department of Biochemistry on this campus, has led to a crystalline derivative that has not yet been obtained in an analytically defined form. Its identity, at present, is concluded from the mode of synthesis and from the general properties of the product.

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DERIVATIVES OF MENADIONE AS ELECTRON ACCEPTORS IN THE OXIDATION OF REDUCED DIPHOSPHOPYRIDINE NUCLEOTIDE

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The results of recent reports suggest a relationship between naphthoquinones and electron transport. Martius and collaborators[1-3] observed a stimulation of electron transport and oxidative phosphorylation by vitamin K_1 and an uncoupling of oxidative phosphorylation by 3,3′-methylenebis-(4-hydroxycoumarin) in studies of animal tissue preparations. Wosilait and Nason,[4] Dolin,[5] and Cormier and Totter[6] reported an acceleration of the rate of oxidation of reduced diphosphopyridine nucleotide (DPNH) by 2-methyl-1,4-naphthoquinone (menadione) in protein preparations obtained from microörganisms. According to a report by Uehara and Muramatsu,[7] the oxidation of phosphogluconate, glucophosphate, and ribose phosphate by yeast autolysates is promoted by menadione. Ball *et al.*[8] observed an inhibition of the reduction of cytochrome c by 2-alkyl-3-hydroxynaphthoquinones and suggested that the inhibition occurred between cytochromes b and c. The inhibition of the reduction of cytochrome c by 2,3-dimercaptopropanol (BAL) observed by Slater[9] and by antimycin A shown by Chance,[10] and the uncoupling of oxidative phosphorylation by 3,3′-methylene bis-(4-hydroxycoumarin),[1] are suggestive of inhibition of a factor or factors which function in electron transport. The inhibitors BAL,[9] antimycin A,[10] and the 2-alkyl-3-hydroxynaphthoquinones[8] appear to exert their effects at the same site.

Chance[11] found that the cytochrome b content of Ehrlich, Krebs 2, and dba thymoma ascites tumors was markedly low as determined by spectroscopic measurement. Strength and Seibert[12] reported that the addition of cytochrome b and menadione to preparations of lymphosarcoma 6C3HED markedly increased the rate of cytochrome c reduction by DPNH, while cytochrome b or menadione alone was ineffective. Dicoumarol inhibited cytochrome c reduction under these conditions and the inhibition was reversed by further additions of menadione. The site of stimulation of the reduction of cytochrome c by menadione and the inhibition by dicoumarol was localized between cytochromes b and c. Reif *et al.*[13] and Reif and Potter[14] in studies of certain normal and tumor tissues obtained results suggestive of antimycin-sensitive and antimycin-insensitive pathways of DPNH oxidation. All the tissues studied exhibited DPNH oxidation